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ONTARIO RESEARCH ENHANCEMENT PROGRAM (O R E P)

Final Report

Improved Quality and Storability of Onions and Carrots Through Sustainable Management of Soil-borne Diseases

REPORT No. OREP - 1999/25

LEAD INVESTIGATOR
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Canada

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Improved Quality and Storability of Onions and Carrots Through Sustainable Management of Soil-borne Diseases

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FORWARD

The Ontario Research Enhancement Program (OREP) is a $4-million two-year federal research initiative that is administered by the Research Branch of Agriculture and Agri-Food Canada (AAFC), with input from the agriculture and agri-food sector, universities and the province. Research focuses on two areas identified by the sector as:

1. Priorities responding to consumer demand for higher quality products; and
2. Ensuring crop-production management systems are environmentally sustainable.

Biotechnology is one of the areas to be explored. Ontario's agricultural production is based primarily on growing diversified crops using intensive production practices, but long-term viability is linked to the development of sustainable crop production management systems.

There are also opportunities to enhance the economic contribution of Ontario's agriculture and agri-food sector by adding value to the diversified primary commodities produced in the province. The Program is expected to be of particular interest to the corn, soybean, greenhouse, fruit and vegetable sectors and the emphasis will be on projects focussing on food quality improvement and sustainable crop production management such as:

1. The development of value-added animal and crop food products and ingredients that meet domestic and export market demands, with a particular emphasis on quality issues such as processing, packaging, shelf life, new product and ingredient development, high-value alternative uses, probiotics and functional foods;
2. Biotechnologies to reduce environmental pressures related to crop protection practices; and,
3. Integrated crop protection and production practices for both field and greenhouse crops.

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EXECUTIVE SUMMARY

Onions and carrots are the major crops grown on muck soils in Ontario and , as a result, the production of these crops is closely associated on many farms, particularly in the system of crop rotation. In general, these are excellent rotation crops, with very few of the same insects and diseases. However, the crop rotation systems currently in place do not address the problems of soil-borne diseases that persist in the soil for long periods. Improving the management of these diseases would reduce crop losses, and increase crop quality and the economic stability of vegetable production.

Two fungal diseases, white rot of onion, caused by *Sclerotium cepivorum* Berk, and sclerotinia rot of carrot, caused by *Sclerotinia sclerotiorum* (Lib.) De Bary, threaten the production of onions and carrots, respectively. Onion white rot causes large crop losses in the field and in storage, especially in cool and wet growing seasons. Sclerotinia rot is the major cause of carrot losses in cold storage, although the disease starts in the field. White rot, in particular, threatens the crop rotation system in muck soil areas because *S. cepivorum* persists as sclerotia, which can survive in soil for 15-40 years. Sclerotia of *S. sclerotiorum* also may survive for extended periods of time (1-6 years) in soil (Adams and Ayers, 1979). Moreover, this fungus has such a wide host range that only a few crops (such as onions) are effective rotation crops for disease reduction. Thus, integrated approaches that reduce both soil-borne diseases, will improve the efficacy of crop rotation and other disease management practices, and will minimize losses in both crops.

Field and laboratory trials were conducted on soil-borne diseases of onions and carrots to evaluate and identify effective methods of disease management, and to determine the effect of these practices on the subsequent crops.

The application of diallyl disulfide (DADS), a synthetic material that stimulates the germination of sclerotia of *S. cepivorum*, was very effective in reducing the incidence of white rot in onions. Two applications prior to onion cultivation were more effective than a single application. DADS is considered bio-rational by the American Environmental Protection Agency (EPA) and is currently in the registration system in the United States. Once it is registered the US, United Agri-Products intends to apply for registration in Canada. Another product, n-dipropyl disulfide, was somewhat effective in reducing the incidence of onion white rot after two applications.

Host resistance to disease is a preferred method of disease management because it is environmentally friendly. Since few or no fungicides need to be applied to crops with disease resistance, this approach is usually very cost effective, once the plant breeding and development has been completed. Field trials demonstrated differences in resistance of onions to white rot,

although the levels of resistance were not sufficient to meet industry's requirements for field use. However, the identification of resistance has allowed commercial plant breeders to work on improving resistance levels in commercially acceptable onions.

An efficient laboratory method of pre-screening onion lines for the levels of resistance to white rot has been tested. Differences among breeding lines could be detected using this method, which is much faster and often more reproducible than field trials.

Biological controls also have the potential to provide environmentally friendly disease management. However, the *Trichoderma harzainum* based commercial bio-fungicidal products, T-22 RootShied™ Drench and T-22 RootShied™ Granular (BioWorks Inc., Geneva. NY, USA) were not effective in reducing the viability of sclerotia of *S. cepivorum*, under the test conditions.

Sclerotinia sclerotiorum infects carrots while they are in the field, but often symptoms of the disease are not evident until the carrots are in long term cold storage. In storage, the disease can cause losses up to 100%. There has been considerable research on sclerotinia diseases of other crops, but little work has been conducted on the epidemiology of this disease on carrots. A solid understanding of the biology and disease cycle is key to improving disease control in the field and in storage.

Two important stages in the disease cycle were identified in this study. Old and dying leaves in contact with moist soil appeared to be the most important infection target in the above ground portion and later the infection was transmitted into the carrot root. Also, senescent decaying leaves present during the growing season and after harvest may contribute to the production of overwintering inoculum. The sequence of events that leads to the infection of the leaves in the field was also determined. Inoculum, in the form of ascospores from neighbouring fields, appears to be important in initiating disease. *Trichoderma harzainum* based commercial bio-fungicidal products, T-22 RootShied™ Drench and T-22 RootShied™ Granular were not effective in reducing the viability of sclerotia of *S. sclerotiorum* and reducing sclerotinia rot on stored carrots, under the test conditions.

Laboratory trials confirmed that storage of carrots at or near 1 °C was effective at suppressing the development of sclerotinia rot in storage. However, it is not always possible for growers to maintain optimum conditions in commercial storage facilities. When storage temperature were increased, sclerotinia rot developed rapidly and no treatments were effective in suppressing the disease.

The most effective method of controlling onion white rot involved treating the fields twice with DADS, at yearly intervals before growing onions. The production of cut-and-peel carrots in the treated fields would fit nicely into the crop rotation system, since these carrots require a shorter growing season than packaging or processing carrots. The DADS could be applied in the spring or early fall, before or after the carrot cropping season. Trials were conducted to determine if there were DADS residues or an off flavour as a result of the treatments. No residues or negative effects on carrot flavour were detected, indicating that this crop rotation system could be recommended once DADS becomes registered.

This report was prepared by graduate students, Maria Jamie and Cezarina Kora, and researchers Dr. Mary Ruth McDonald and Dr. James Traquair, with input from Marilyn Hovius and Kevin Vander Kooi.

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INTRODUCTION

Crop rotation is an integral and very basic component of Integrated Pest Management and sustainable agriculture. On both mineral and organic soils, crop rotations with onions and carrots have successfully reduced losses caused by disease, insects, nematodes and weeds, since these crops share almost none of the same pests. At present, this system of crop rotation is breaking down, especially on organic soils, primarily because of two soil-borne diseases: white rot of onion and sclerotinia rot of carrots, lettuce and celery.

Sclerotium cepivorum Berk, the causal agent of onion white rot (Fig.A), persists in the soil as sclerotia which can survive 15-40 years in the absence of a host (Coley-Smith, 1990). The chemical fungicide Botran is registered for white rot control, however it is expensive and only partially effective (McDonald *et al.*, 1992). Thus, growers avoid growing onions in fields known to be infested with *S. cepivorum*. Other important muck vegetables such as carrots, celery and lettuce can be grown in these fields and will not be infected by *S.* cepivorum. However, these crops are all susceptible to *Sclerotinia sclerotiorum*.

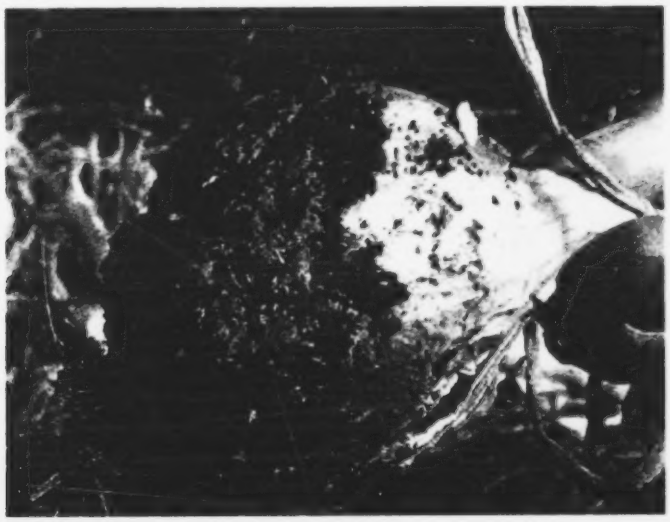

Figure A. White rot of onion, caused by *Sclerotium cepivorum* Berk.

Sclerotinia rot of carrots (Fig. B), caused by *S. sclerotiorum*, is the major cause of carrot losses in storage. After a maximum of three consecutive years of growing any one of the susceptible vegetable crops in the same field, crop losses due to sclerotinia rot infection can be prohibitive. *S. sclerotiorum* also persists in the soil as sclerotia, but these are reported to survive only two to three years in the absence of a host (McDonald, 1994). Under current practices in Ontario, three years of rotation out of a susceptible crop does not appear to be sufficient to reduce disease.

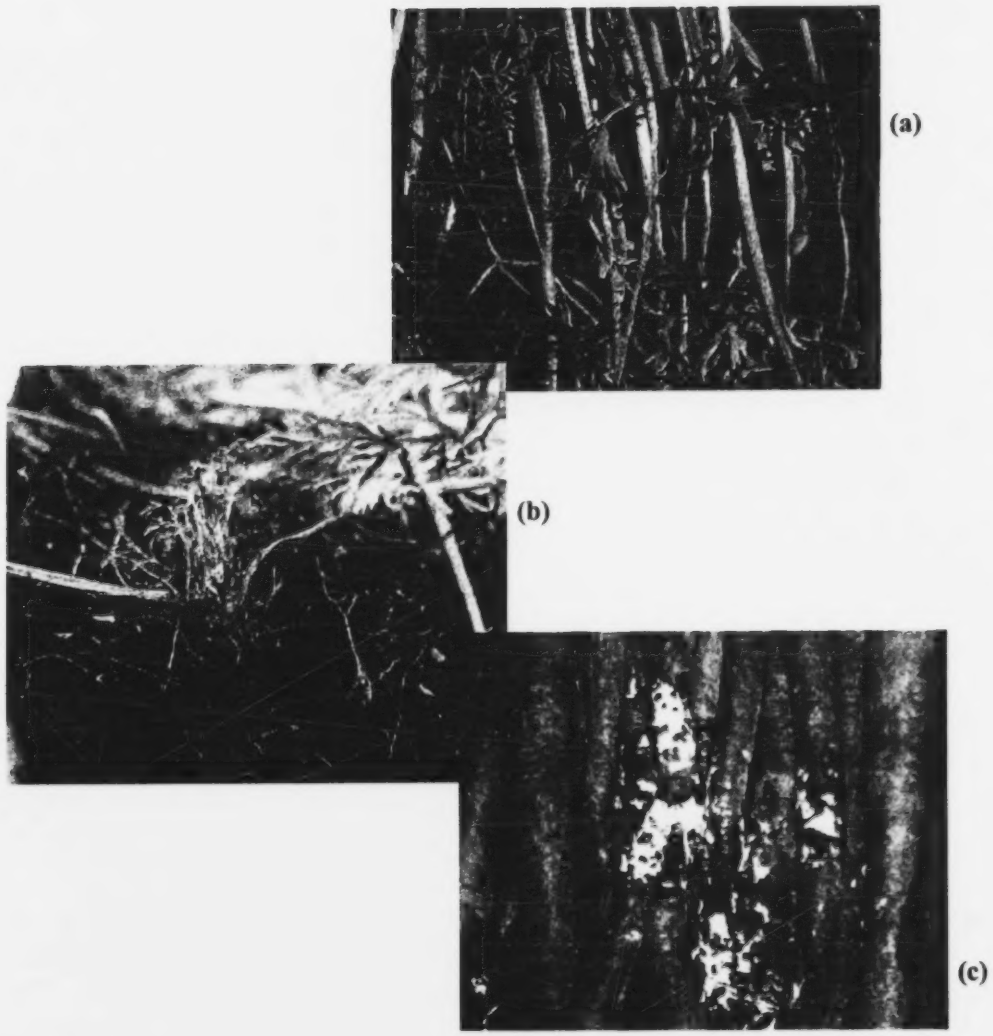

Figure B. Sclerotinia rot of carrot, caused by *Sclerotinia sclerotiorum* in the field (**a** & **b**) and storage (**c**)

The detrimental effects of these two diseases can easily be seen in the Bradford area, but *Sclerotinia* diseases threaten the crop rotation systems in many other parts of Ontario. As onion white rot spreads to more fields in the Bradford area, there is less opportunity for crop rotation, and losses due to both soil-borne diseases are increasing. Both of these pathogens initially infect the host crop in the field, but significant losses occur while the crop is in storage over the winter.

Onions infected with white rot may develop symptoms in the field, especially in the late summer (Chaput, 1994). However, onions with no visible symptoms at harvest may develop the disease while in cold storage or in transit to markets. Ontario onions are known for their exceptional keeping quality. If onions are suspected of white rot infection, they are not acceptable for export. Exporters cannot take the risk that disease will develop on the onions while in transit. The Ontario industry must export 20-40% (0.8-1.6 million bushels) of the onions produced each year to avoid a local glut and concomitant low prices. The ability to produce large volumes of export quality onions is becoming even more important as the Bradford and District Vegetable Growers Association begins a "Grow Ontario"- funded promotion of Ontario "Picante" onions in the southern United States.

A number of approaches have been tested for the management of white rot. Flooding in conjunction with crop rotation provided some reduction in white rot incidence (Banks and Edgington, 1989), however flooding is not possible in many areas and crop rotation alone is ineffective (Banks and Edgington, 1989). To-date, application of the sclerotial germination stimulant diallyl disulfide (DADS) is the most promising control method (Crowe *et al.*, 1991). Biological control of *S. cepivorum* with microparasites such as *Trichoderma harzianum* has also been successful (Leggett and Rahe, 1985). Until recently, the commercial availability of bio-control agents has been a problem but a new strain of *T. harzianum* (T-22) has now been registered in the United States (BioWorks, Inc., Geneva, NY, USA).

Control of onion white rot would increase marketable yields and the proportion of export quality onions. It would also decrease the cost now associated with grading of onions, which occurs up to three times during the winter. Increased export of onions would be possible along with more orderly marketing of the onion crop. There would be more high quality onions that could keep until May or June, supplying the local markets for a longer period and reducing the import of onions into Ontario.

The ability to store carrots for several months during the winter is essential to the Ontario carrot industry, which also depends on export markets. The development and spread of Sclerotinia rot is the most important limiting factor to long term storage of carrots. Losses have been increasing over the past 20 years, probably as a result of increasing inoculum density in the soil, as well as the loss of Benlate (benomyl) as a post-harvest dip. In years where *S. sclerotiorum* is prevalent, growers are forced to sell their carrots early to avoid further losses in storage and more carrots are imported from the United States.

Problems with quality and storability of carrots promise to become a serious issue as the Ontario industry starts to produce cut-and-peel carrots (the "baby" carrots sold in cello bags). This industry has revolutionized carrot production in the United States and increased carrot consumption there by 25%. Most of these carrots now available in Ontario are from California, but local production of this commodity has started, spearheaded by Gemini Foods and a small support industry in the Bradford area. For the Ontario industry to be viable and to use Ontario carrots to the greatest advantage, high quality Ontario carrots must be available throughout the year.

Sclerotinia rot disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is the most destructive and most economically important disease of cultivated and stored carrots (*Daucus carota* L.) worldwide (Lewis & Garrod, 1983; Finlayson *et al.*, 1989; McDonald, 1994). The infection originates in the field but most disease development occurs during storage. Often disease symptoms are not visible at harvest. Disease development in storage can substantially affect the marketable yield and storage life of carrots. Economic losses of 80-100% attributed to *S. sclerotiorum* have been reported. This pathogen is a widely distributed soil-borne fungus with a host range consisting of at least 408 species (Boland and Hall, 1994), including numerous economically important vegetables, field crops and flowers.

There are about 3000 ha of carrots grown in Ontario. This province is one of the few places in the world where over half of the crop is stored for up to 2 to 6 months. Almost half of the Ontario's carrots are grown in the organic soils of the Bradford Marsh area where most of the carrot fields are infested by *S. sclerotiorum. S*clerotinia rot is becoming a major limiting factor to the long-term storage of the crop. There is only one fungicide (Benlate), registered for field treatments to control sclerotinia rot of carrot, however this provides only partial control and not economically acceptable levels of control.

T. harzianum has been reported to have antagonistic and mycoparasitic effects against sclerotia of *S. sclerotiorum* (Steadman, 1979; Budge & Whipps, 1991, Garcia-Garza *et al.*, 1997) and several other soil-borne fungi including *Pythium* spp., *Rhizoctonia* spp. *S. homeocarpa* and *Sclerotium* spp. No research has been conducted to evaluate its use in the carrot-sclerotinia pathosystem. Tronsmo (1989) reported that storage rot of carrots caused by *S. sclerotiorum* was reduced during long term storage at 0^0C by dipping the roots after harvest in a conidial suspension of a cold-tolerant strain of *T. harzianum*. *T. harzianum* is a naturally occurring soil fungus, and beside its potential as a bio-control agent, it is well recognized as a growth promoter, plant performance enhancer and inducer of plant's self-defence mechanisms (Kleifeld & Chet, 1992).

PROJECT OBJECTIVES

1. **To improve the management of onion white rot by:**

   1.a Evaluating the bio-control agent *Trichoderma harzianum* for the control of onion white rot in the field;

   1.b Conducting field trials to assess the efficacy of DADS for reducing onion white rot (continuation of trial);

   1.c Assessing the survival of sclerotia of *S. cepivorum* exposed to DADS and *T. harzianum*;

   1.d Determining if artificial curing reduces white rot development or spread in storage;

   1.e Further investigating the potential for development and spread of white rot in storage;

   1.f Evaluating onion breeding lines for resistance to white rot in greenhouse trials (method of Entwistle, 1993) and relating this to field plot data from the 1997 trials.

2. **To improve the management of sclerotinia rot of carrot by:**

   2.a Determining the potential of calcium nitrate, Nitro- 9 Plus, calcium propionate, sodium bicarbonate and lime as field applications or post-harvest dips on the development of *S. sclerotiorum* on carrots suitable for cut-and-peel processing, in storage, with and without hydro cooling;

   2.b Conducting laboratory trials on the development and spread of sclerotinia rot on carrots treated as above;

   2.c Evaluating the biocontrol agents *Coniothyrium minitans* and *Sporodesmium sclerotivorum* for the control of sclerotinia rot of carrots;

   2.d Assessing the survival of sclerotia of *S. sclerotiorum* exposed to *C. minitans* and *S. sclerotivorum*;

2.e Obtaining weather and disease incidence data to contribute to the understanding of the epidemiology of sclerotinia rot on carrots;

2.f In conjunction with a concurrent project on production of cut-and-peel carrots, evaluate the effects of pre and post-harvest treatments on quality and shelf-life, with and without specialized packaging.

3. To assess the potential for integrating the control methods in onion and carrot production by:

3.a Determining the potential to integrate DADS applications with cut-and-peel carrots cropping system by applying DADS before carrots are seeded and again shortly after harvest, and determining DADS residues in the carrots;

3.b Investigating the effects of the white rot bio-control agent *T. harzianum,* on survival of sclerotia of *S . sclerotiorum* and the effect of *C. minitans* and *S. sclerotivorum* on the survival of sclerotia of *S. cepivorum* (Field and greenhouse trials).

MATERIALS AND METHODS

1. Management of onion white rot

1.a. Evaluation of the bio-control agent *Trichoderma harzianum* for the control of onion white rot

Two commercial biological fungicide formulations, T-22 RootShield™ Drench (T-22RSD) (*Trichoderma harzianum* Rifai strain KRL-AG2 1.15%, $1x10^7$ colony forming units per gram dry weight) and T-22 RootShield™ Granular (T-22G) (*Trichoderma harzianum* Rifai strain KRL-AG2 1.15%, $1x10^7$ colony forming units per gram dry weight), (BioWorks Inc., Geneva, NY, USA), were evaluated for the control of white rot in onions.

Onion seeds (cvs. Fortress and Hustler 1997. cvs. Fortress and Frontier, 1998. cv. Fortress, 1999) were treated with Pro Gro at 25 g of product with 1% methyl cellulose per kg of seed. The onions were seeded into organic muck soil (65% organic matter, pH 5.5-6.5) naturally infested with the pathogen in commercial fields in the Holland Marsh, Ontario. The onions were seeded

using a V-belt push seeder delivering 39 to 46 seeds/m at a depth of 1.5 to 2.0 cm. A treatment of T-22G was applied on the V-belt along with the seed (Table1.a.1). The T-22 RSD treatments were applied using a gravity flow line placing the drench directly in the seed furrow. See Table 1.a.1. for rates.

A randomized complete block design with four replications per treatment and cultivar was used. Each replicate consisted of one row 3 m in length. Recommended control procedures for other fungal and bacterial pathogens, weeds and insects were followed according to OMAFRA Publication 363. The crop was managed for the entire season by the grower. Onion bulbs (120 approx.) were assessed for white rot incidence at maturity.

Table 1.a.1. Summary of cultivar, treatments and dates for year.

Year	Cultivar	Treatment date ProGro	Seed date	Assessment date	Treatments
1997	Fortress Hustler	4 June	5 June	14,15 Oct	1) T-22RSD at 2.34 g/100 m of row in 1500 L of water/ha (low rate) 2) T-22RSD at 4.67 g/100 m of row in 1500 L of water/ha (high rate) 3) T-22G at 46.7 g/100 m of row 4) Untreated control
1998	Fortress Frontier	11 May	15, 22 May	19,21 Sept	1) T-22G at 44.8 g/100 m of row, 2) T-22RSD at 4.48 g/100 m of row in 1500 L of water/ha (low rate) 3) T-22RSD at 22.5 g/100 m of row in 1500 L of water/ha (high rate) 4) Untreated control
1999	Fortress	10 May	11, 12, 20 May	2,6 Oct	1) T-22G at 120 g/100 m of row (low rate) 2) T-22G at 240 g/ 100 m of row (high rate) 3) T-22RSD at 40 g/100 m of row in 1500 L of water/ha (low rate) 4) T-22RSD at 80 g/100 m of row in 1500 L of water/ha (high rate) 5)Untreated control

1.b. Field assessment of the efficacy of DADS for reducing onion white rot

Two synthetic germination stimulants, DADS (diallyl disulphide 85.5%, diallyl sulphide 4.5%) and DPDS (n-propyl disulphide 88%, related compounds, 2%). obtained from United Agri-Products (Dorchester, Ontario, Canada), were applied to commercial field plots (65% organic matter, pH 5.5-6.5) naturally infested with the pathogen in the Holland Marsh, Ontario. These sites had been treated with DADS and DPDS twice, except site 3 which was treated once during 1996 (Table 1.b.1). The field plots were located in seven different farms with a previous history of white rot. All trials were randomized complete block designs with 6 replications per

treatment (approximately 2.5m x 16m). The replications were separated by a minimum of 0.3 m in all trials. Applications were made after the grower finished harvesting the crop at the particular site, and once the maximum soil temperature at 10cm depth remained below 21 C for several days in order to avoid the possibility of soil temperatures exceeding 24 C at any time in the fall after treatment. The two products, DADS and DPDS were applied to depths of 10 and 20 cm using a modified Vorlex soil fumigator with eleven injection hoses spaced 20 cm apart at a rate of 10 L of product/ha in 500 L of water/ha. The plot areas were sealed following treatment, using a mechanical roller and the soil remained undisturbed until spring. In the spring, the grower seeded the sites and managed the onions for the full season. Recommended control procedures for other fungal and bacterial pathogens, weeds and insects were followed according to OMAFRA Publication 363. At harvest, approximately 50 onions, in each of four subplots (1.22 m x 2m per subplot) per replication, were assessed for incidence of white rot, for a total of approximately 200 onions per replication.

Table 1.b.1 Summary of treatment application rates and dates at field sites using synthetic germination stimulants of sclerotia: diallyl disulfide (DADS) and di-N- propyl disulfide (DPDS).

Site	Germination stimulants	Rate in 500L of water/ha.	Application dates	# of applications
1	DADS	10L/ha	21 Aug '95 &19 Sept '96	Two
	DPDS	10L/ha	21 Aug '95 &19 Sept '96	Two
2	DADS	10L/ha	21 Aug '95 &19 Sept '96	Two
3	DPDS	10L/ha	21 Aug '95 &19 Sept '96	Two
3	DADS	10L/ha	19 Sept '96	One
4	DADS	10L/ha	19 Sept '96 & 24 Sept '97	Two
5	DADS	10L/ha	19 Sept '96 & 24 Sept '97	Two
6	DADS	10, 5 L/ha	2 Oct '98 & 30 Jun '99	Two
7	DADS	10, 5 L/ha	2 Oct '98 & 17 May '99	Two

1.c. Assessment of the survival of sclerotia of *Sclerotium cepivorum* exposed to DADS

Nylon mesh sacks (5x5 cm) of 100 sclerotia of *S. cepivorum* (laboratory cultured, note below) were placed in plastic pots (15 cm depth) of organic soil (65% organic matter, pH 5.5-6.5, from the Muck Crops Research Station) treated with DADS (diallyl disulfide 85.5%, diallyl sulfide 4.5%) and DPDS (di-N-propyl disulfide 88%, related compounds 2%). The pots were placed in the greenhouse and recovered at monthly intervals(1, 2 and 3 months). The pots were arranged in a randomize complete block design with 4 replications per treatment. The soil was

kept moist during the experiment by watering when necessary. Temperature in the greenhouse ranged between 10 to 25 °C. Intact sclerotia were extracted (see below extraction process), surface sterilized (1 minute in 75% Ethanol, 3 minutes in 10% bleach solution, 3 minutes in sterile water twice) and plated on potato dextrose agar (PDA) to test for viability. They were incubated at 20-21°C for 28 days and were examined at 7, 14, 21 and 28 days after plating. The germinated sclerotia were counted as viable, confirmed with the later formation of new sclerotia. In 1997, 1998 and 1999, similar sacs were buried 10 cm deep in the treated and non-treated field plots shortly after treatment (muck soil 65% organic matter, pH 5.5-6.5) (All Sites, Table 1.c.1) and recovered at monthly intervals for 3 months. Remaining sclerotia were counted and tested for viability as above. Mortality of sclerotia was calculated and assessed as (1) loss, and (2) loss plus extraction of non-viable lab assessment of mortality, and (3) mortality (percent) (Table 1.c.2).

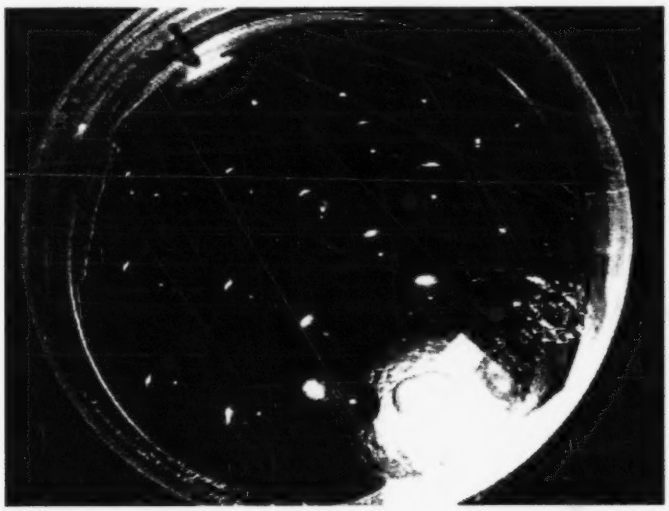

Figure 1.c.1. Sclerorial viability test, on PDA drop plates

Sclerotia culture

Sclerotia from field samples were collected and surface sterilized (1 minute in 75% Ethanol, 3 minutes in 10% bleach solution, 3 minutes in sterile water twice), blot dried and plated into petri dishes containing potato dextrose agar (PDA). After 14-16 days of incubation, the plates were full of sclerotia. The sclerotia were harvested and stored in a glass jar for three months at room temperature (21 to 23 °C), to pass the dormancy period (Coley-Smith *et al* 1987).

Once the three months had passed, a viability test was performed. One hundred sclerotia were surface sterilized and plated onto PDA drop plates, with 15 droplets per plate. They were incubated at 20-21°C for 28 days. Evaluations of the viability (7, 14, 21, 28 days) were made by visual assessment of the germination of the sclerotia, based on the presence of white fluffy mycelium and confirmed with the later formation of new sclerotia.

Sclerotia extraction from sacks

The dirt of the nylon mesh sack was washed away with tap water. The sclerotia were pushed to one corner of the sack, using the current of the tap water. Subsequently, the sack was divided in half (diagonally) and the sclerotia were placed into a petri dish and surface sterilized.

Table 1.c.1. Summary of pot trials and field trials to assess sclerotia survival

Site	Germination Stimulants	Rate in 500L of water	Monthly interval
Pot trial 97	DADS	10 L/ha	1, 2, 3 months
	DPDS	10 L/ha	1, 2, 3 months
Site 1-5 field trials*	DADS	10 L/ha	3 & 7 months
Site 6-7 field trials*	DADS	5 & 10 L/ha	1, 2, 3 months

* Sacks placed in sites treated for field assessment of the efficacy of DADS for reducing onion white rot (section 1b)

Table 1.c.2. Calculations for sclerotia mortality after exposure to field sites treated with the synthetic germination stimulants: diallyl disulfide (DADS) and di-N-propyl disulfide (DPDS).

Dependent variable	Calculation
1. Loss	[(Total # sclerotia exposed to treatment* - total number recovered post-treatment)/100]x100=% loss
2. Field loss + lab assessed mortality	[(100 - total number recovered post-treatment) + (total number recovered - total number germinated in culture post-treatment)]/100]x100=% mortality
3.Mortality	[(100 - total number recovered post-treatment) + (total number recovered - post-treatment)]/100 - total number contaminated in culture]x100=% mortality

*Total number of sclerotia exposed to soil treatments was always 100.

Note: Objectives **1.d** and **1.e** were not accomplished, details were provided in the interim report.

1.f Evaluation of onion breeding lines for resistance to white rot in greenhouse

A) Scale inoculation (Method of Entwistle 1993)

Scale segments from harvested yellow cooking onion bulbs of commercial cultivars and breeding lines (obtained from Dr. I.L. Goldman at the University of Wisconsin, Petoseed, Asgrow Ltd and commercial cultivars) were grown in the Holland Marsh, Ontario, in a commercial field in 1997-1999. Scale sections from the onion bulbs were inoculated with mycelial plugs (7 days old) of isolates of *S. cepivorum*. Two isolates of *S. cepivorum* Berk, mycelial compatibility MCG-1 and MCG-2 were used in 1997 & 1999, and three isolates MCG-1, MCG-2 and MCG-3 were used in 1998. The scale segments were prepared for inoculation as follows: bulbs were surface disinfested, after removal of the outer scales, in a 10% hypochlorite (commercial Javex bleach) solution (5 minutes), rinsed with sterile water and air dried as needed. Scale segments (7 cm x 7 cm) were cut, the inner membrane was removed and the scales were aseptically inoculated. Agar discs, 5 mm in diameter, were cut from the margins of 7 days old actively growing cultures, using a sterile cork borer and placed mycelium side down in the centre of each segment (concave side). Each mycelial line was replicated four times in a randomized complete block design. Each replication was placed in sterilized (wiped with 75% alcohol and 10% hypochlorite) plastic trays (28x54cm) and stacked in a plexiglass chamber (155x59x65 cm) which was filled with water to 7.5 cm. A hydro-thermograph was placed inside and the chamber was covered with a black sheet. After 7 days incubation at room temperature (21 to 23 °C) the lesion diameter on each scale (convex side) was measured.

B) Field trials

Field resistance to white rot was investigated at one commercial field site with a history of white rot (muck soil, 65% organic matter, pH 5.5-6.5) and in an outdoor pot trial at the Muck Crops Research Station, Holland Marsh, Ontario in 1998. Onion lines from four sources (University of Wisconsin, Petoseed, Asgrow and Sun Seeds) and commercial cultivars were seeded in 288 plug trays, filled with Sunshine mix, and hand-transplanted, after 6 weeks of growth in the greenhouse, in the commercial field site and the pot trial. Each cultivar was replicated four times in a randomized complete block design. Each replicate in the field trial consisted of one 3 m row, at 40 plants/m with 42 cm between rows. In the pot trial, each replicate consisted of one plastic pail (30cm in diameter, 36cm deep). White rot free organic muck soil was infected with sclerotia of *S. cepivorum* at 300 viable sclerotia per kg of soil (tested on potato dextrose agar) and mixed with a hand trowel (5 minutes). Seven plants were transplanted per replication, spaced 3 cm apart. Recommended control procedures for other fungal and bacterial pathogens, weeds and insects were followed according to OMAFRA

Publication 363. The plants in the pot trial were watered when the soil appeared dry. Onion bulbs were assessed for white rot incidence at harvest.

Data analysis

Data were analysed using the General Analysis of Variance function of the Linear Models section of Statistix, V. 4.1.

1. Management of sclerotinia rot of carrot

1.a. Assessment of the efficacy of field applied fungicides and calcium nitrate, Nitro-Plus, calcium propionate, sodium bicarbonate, and sodium hypochlorite washes as post-harvest dips on development of sclerotinia rot in cut-and-peel carrots

In 1997, carrots were direct seeded (96 seeds/m) in organic soil (pH 6.4, organic matter 60%) naturally infested with the *Sclerotinia sclerotiorum* at the Muck Crops Research Station on 27 May. A randomized complete block arrangement was used. Each replicate consisted of four raised beds (86 cm apart), 5 m in length. There were two field treatments: Benlate 50WP at 1.1 kg/ha and Bravo 500 at 3.2 L/ha. Both treatments were applied on 15, 23 Sep and 8 Oct as foliar sprays using a pull type plot sprayer with D-3 hollow cone nozzles in 500 L/ha of water at 100 psi (boom). Rainfall and air temperature were recorded and are reported below. A tap water washed check at 15°C and unwashed field check were also included. Carrots were harvested from the two center rows of each plot on 3 and 4 Nov, 1997. Twenty-four bushels (approx. 600 kg) were also harvested on 4 Nov, 1997 from adjacent untreated check plots and were washed and dipped on 5 Nov, 1997 for 30 seconds in one of five solutions: Benlate 50WP at 2.2 g/L water; Bravo 500 at 6 mL/L water; Botran 75W at 3.7 g/L water; Calcimax at 0.1% solution (1.25 mL/L water) and Nitro 9 at 0.1% solution (1.42 mL/L water). An untreated washed check was also included. All treatments were placed in a Filacell storage where the temperature and relative humidity were kept at approximately 1°C and 95% respectively. The number of carrots with and without white mold (Sclerotinia) were counted on 30 Jan, 2 Feb, 1998 and 11 May, 1998. Data were analyzed using the General Analysis of Variance function of the Linear Models section of Statistix V.4.1.

In 1998, carrots were direct seeded (120 seeds/m) in organic soil (pH 6.4, organic matter 60%) naturally infested with *S. sclerotiorum* at the Muck Crops Research Station on 28 May. A randomized complete block arrangement was used. Each replicate consisted of four raised beds

(86 cm apart), 5 m in length. Field treatments were: Benlate 50WP at 1.1 kg/ha at 500 L/ha and 1000 L/ha, Ronalin at 2.25 kg/ha, Calcimax at 5% solution, NITRO 9 at 5% solution, Nova Cal at 1000 kg/ha and 2000 kg/ha A spray treatment of *Coniothyrium minitans* was applied before planting in 1000 L/ha of water, then roto-tilled into the soil before the beds were formed and seeded. A drench treatment of *C. minitans* was applied in 1500 L/ha of water using a Solo back pack sprayer (60psi) without a nozzle. The drench was applied over the entire seed bed right after seeding. *C. minitans* was applied to both treatments at a rate of 1.25×10^{13} spores/ha. The entire trial was irrigated with 2.5 cm of water after seeding. All spray treatments were applied on 17, 23 Sep and 5 Oct as foliar sprays using a pull type plot sprayer with D-3 hollow cone nozzles in 500 L/ha of water at 100 psi (boom). The air temperatures were above the long term (10 year) average for May and not different from the long term average for June, July, Aug and Sept. Total rainfall was below the long term (10 year) average for May (42.6 mm), July (50.2 mm), and Sept (18.6 mm), above average for Aug (114.6 mm) and not different from the long term average for June (78.4 mm). An untreated field check were also included. Carrots were harvested from the two center rows of each plot on 2 and 3 Nov, 1998.

Twenty-four bushels (approx. 600 kg) were also harvested on 3 Nov, 1998 from adjacent untreated check plots and were washed and dipped on 4 Nov, 1998 for 30 seconds in one of six solutions: Benlate 50WP at 2.2 g/L water; Calcimax at 1.0% solution (12.5 ml/L water), Nitro 9 at 0.1% solution (1.42 ml/L water), Nitro 9 at 1.0% solution (14.3 ml/L), Pathoclean at 2.0 ml/L and a hydro-cooling treatment in 4^0C water for 2 minutes. An untreated washed check was also included. All treatments were placed in a Filacell storage where the temperature and relative humidity were kept at approximately 1°C and 95% respectively. The number of carrots with and without white mold (Sclerotinia) were counted and rated for disease on 2 Mar 1999 and 6 Jul 1999. Data were analysed using the General Analysis of Variance function of the Linear Models section of Statistix V.4.1.

1.b. Laboratory assessment of the efficacy of calcium nitrate, Nitro-Plus, calcium propionate, sodium bicarbonate, and sodium hypochlorite washes as post-harvest dips on development of sclerotinia rot in cut-and-peel carrots

Samples of cut-and-peel carrots (cv. Indiana) were dipped in selected treatment solutions for 30 minutes. Treatment solutions were 0.1% calcium nitrate,1% Ca in Nitro-9 stock solution, 1% calcium propionate, 1% sodium bicarbonate, sodium hypochlorite at 100 ppm, and distilled water control. After each dip treatment, five carrots were drained and placed side-by-side (touching) in each of three small plastic crispers (30 x 13 x 10 cm). There were six replicate boxes for each treatment and control. The midpoint of the centre carrot in each box was wound-inoculated with

Sclerotinia mycelium in 1-cm potato dextrose agar disks cut from the margin of 5-day-old cultures or 5-day-old, pure cultures on carrot disks. The carrots were incubated on wire racks about 3 cm above moistened paper liners in these humid plastic crisper boxes randomly positioned on two shelves in walk-in cold rooms for 8 weeks at 1°C and 6 °C. Spread of the pathogen during storage was measured as the width and length of lesions (or colony) including surface mycelium, from the inoculated centre carrot. The means for the three replicates for each treatment was plotted with standard error bars. Data were subjected to ANOVA and differences in treatments were based on Tukey's and Scheffe's Mean Comparison Tests (SPSS Base 7.5 for Windows User's Guide, SPSS Inc., Chicago IL).

2.c Field evaluation of the efficacy of the bio-control agent *Trichoderma harzianum* for the control of sclerotinia rot in field and stored carrots.

Note: The original proposal was to evaluate the efficacy of the bio-control agents *C. minitans* and *S. sclerotivorum* for the control of sclerotinia rot of carrot. However, after 1998 Agrium Inc., who was the supplier of both bio-control agents, discontinued its biological control program. As *C. minitans* and *S. sclerotivorum* were no longer available, we substituted the test material with the mycoparasite *T. harzianum* and evaluated its efficacy for the control of sclerotinia rot in field grown and stored carrots.

Two commercial biological fungicide formulations, T-22 RootShield™ Drench (T-22D) (*Trichoderma harzianum* Rifai strain KRL-AG2 1.15%, $1x10^7$ colony forming units per gram dry weight) and T-22 RootShield™ Granular (T-22G) (*Trichoderma harzianum* Rifai strain KRL-AG2 1.15%, $1x10^7$ colony forming units per gram dry weight), (BioWorks Inc., Geneva, NY, USA), were evaluated to determine efficacy, effective rates and optimum application methods.

Pelleted carrot seeds were direct seeded (90-100 seeds/m) with a precision seeder, in organic soil (pH 6.4, organic matter 60%) naturally infested with *S. sclerotiorum*, at the Muck Crop Research Station, on 27 May 1999. A randomized complete block arrangement with six treatments and four replications was used. Each replicate consisted of four raised carrot beds 86cm apart and 6m long.

The treatments consisted of:

1) Untreated Check
2) T-22D applied as drench immediately after seeding, at 10 kg/ha (86g/100m of row in 1500 L of water/ha);
3) T-22D applied as drench immediately after seeding, at 20 kg/ha (172g/100m of row in 1500 L of water/ha);
4) T-22G applied in the seed furrow, at 30 kg/ha (260g/100 of row),
5) T-22G applied in the seed furrow, at 60 kg/ha (520g/100 of row),
6) T-22D applied as a 5 minutes post-harvest dip treatment of carrot roots, at 8.6 g/100ml water.

Both drench treatments were applied with a watering can with a long extension and perforated nozzle to ensure a gentle spray over a 8cm band of the carrot seed row. The granular treatments were applied with the precision seeder, where two additional hoses directed the product to both sides of the seed furrow at the required rate. The post-harvest treatment was carried out by dipping the sampled carrot roots into a 10 litre freshly diluted solution of the bio-fungicide. Recommended applications for weed and insect control were followed according to OMAFRA Publication 363, Vegetable Production Recommendations 1998-1999. No additional fungicide treatment was applied except for the tested bio-fungicides. Data were analyzed using the General Analysis of Variance function of the Linear Models section of Statistix for Windows ver. 4.1.

A) Root colonization assay

Destructive sampling of two carrot roots from each treatment, was performed during the growing stage (26 July, 26 August) and at harvest (5 November) in order to determine the rate of temporal root colonization by *T. harzianum*. Carrot roots were shaken to remove the extra soil residues. Three root disks of about 1cm each, were cut from the top, middle and the bottom sections of the taproot and plated in Sclerotinia Selective Medium (SSM) consisting of 39 g Potato Dextrose Agar (PDA), 25 g Pentachloronitrobenzene (PCNB), 150 g Penicillin, 150 g Streptomycin sulphate and 50 g Bromophenol blue per 1 liter of distilled water (Steadman 1994). This procedure aimed at simultaneously detecting infection by *S. sclerotiorum* and the presence of *T. harzianum* and was repeated for every replicate. The carrot disks that developed mycelial growth of *T. harzianum*, were counted and percent colonization was calculated for the total number of plated units.

B) Sclerotinia rot development in storage

The effect of field and post-harvest dip applications of *T. harzianum* on control of sclerotinia rot of carrot in storage, were assessed by monitoring the progress of sclerotinia rot disease incidence and severity in storage over time. Approximately 25 kg of carrot samples were harvested from each replicate on 5 November. After the tops were removed, both unwashed carrots from the field treatments and the washed carrots from the post-harvest treatment, were divided into 3 separate polyethylene net bags. As the disease pressure in the field was very low, to insure disease occurrence in storage, a fully colonized carrot root piece of about 5 cm, which was previously inoculated with a 3-day-old mycelial agar plug (7mm in diameter) of *S. sclerotiorum* was introduced into each carrot bag. Carrots were stored in cold temperature controlled Filacell storage at 1.0 ±1.0 ^{0}C and 90 ± 5% RH, immediately after inoculation. The carrots were assessed for incidence of sclerotinia rot and disease severity, in two months intervals for over a period of six months (5 February, 5 April, and 5 June), from the time of artificial inoculation. One bag of carrots was evaluated at each assessment interval. Disease incidence was determined as the percent of infected carrots roots. To estimate disease severity, a disease index was calculated based on a 5 classes severity scale which accounted for the lesion coverage of every infected root: class 1: healthy; class 2: 1 - 25% infected; class 3: 26-50% infected; class 4: 51-75% infected; class 5: 100% infected). The following modified formula of Kobriger and Hagedorn (1983) was used to calculate the disease index:

$$\text{Disease severity index} = \frac{\sum (\text{Severity class} \times \text{Number of roots in that class})}{\text{Total number of roots} \times \text{number of classes}} \times 100 \qquad (1)$$

C) Marketable yield assessment

For the yield assessment, a section of 1.16m of carrot row was randomly harvested from the two center rows of each replicate. The tops were removed, and carrot roots weighed prior to and after the unmarketable (forked and split) roots were graded out. The weight was then converted to yield units (tonnes/hectare) based on the following formula: Yield (T/Ha) = Total sample weight (Kg/Ha) x 10.

D) Cavity spot assessment

For the cavity spot assessment, a sample of 20 consecutive carrots was harvested randomly from the two center rows of each replicate, on 5 November. After the tops were removed the

carrot roots were washed in running tap water, air-dried and assessed for cavity spot incidence and severity. Disease incidence was calculated as the percent of carrot roots in the sample with one or more cavity spot lesions. A disease severity index was calculated based on the size of the largest lesion on the carrot root (measured as horizontal length) and a severity scale of 5 classes as follows: very light <1 mm; light 1-2 mm; medium 2-5 mm; heavy 5-10 mm; very heavy >10 mm.The disease index was calculated using equation (1).

2. d Field assessment of the survival of sclerotia of *S. sclerotiorum* exposed to the mycoparasite *T. harzianum*

To determine the temporal colonization, recovery and viability rate of sclerotia of *S. sclerotiorum* exposed to *T. harzianum* over time, six nylon mesh sacs (5x5 cm) containing 30 sclerotia each, were buried 5 cm deep into the soil in each replicate, two weeks after treatment application (10 June 1999). The sacks were recovered at two week time intervals over a period of 12 weeks, starting from the burial day (24 June, 08 July, 22 July, 5 August, 19 August and 2 September). At each recovery, the sclerotia that were physically intact were surface sterilized (1 minute in 75% Ethanol, 3 minutes in 10% bleach solution, 3 minutes in sterile water), blot dried and plated onto petri dishes containing SSM. They were incubated at 20-21^{0}C for 7-10 days. The germinated sclerotia were counted as viable (confirmed by the change of the color of the media from blue to yellow and new sclerotia formation) and the ones that developed mycelial growth of *T. harzianum*, were counted as colonized. Percent colonization, recovery and viability were calculated for the total number of sclerotia buried. Data were analyzed using the *Proc Glm* (General Linear Model) procedure of SAS System for Windows ver. 6.12 (SAS Institute, Cary, NC, USA). The Type I error rate (α) was set at 0.005 for all statistical tests.

2. e Monitoring of micro-climate, pathogen and disease incidence data for the epidemiological study of sclerotinia rot of carrot.

The epidemiological study was conducted during the summer of 1999 at two sites in Bradford Holland Marsh: Site 1 was in a field at the Muck Crop Research Station on 27 May and Site 2 was located in a commercial carrot field seeded on 15 May. Both fields had a long history of sclerotinia rot on carrot. No fungicide treatments were applied to the carrot crop at Site 1, while fertilizer, herbicide and insecticide treatments were applied according to the standard maintenance program of the station. The experimental plot in the commercial field was managed by the grower and applications for disease, weed and insect management were followed according to OMAFRA Publication 363, Vegetable Production Recommendations 1998-1999. Standard treatments for management of sclerotinia rot of carrot included regular Benlate applications (27 August, 10 September and 27 September).

The packaging carrot cultivar Cello-Bunch was used in all experiments.

A) Monitoring micro-climate variables

Continuous monitoring and records throughout the growing season in both experimental sites, were obtained for the following environmental variables: air temperature, relative humidity, air temperature within the canopy, soil temperature, soil moisture (v/v), rainfall, and leaf wetness duration of exposed and non-exposed leaves. Soil measurement readings were obtained from the top 5 cm of soil layer as this is the maximum depth of occurrence of sclerotia which are capable of carpogenic germination and thus important for the epidemiology of the disease. Atmospheric measurement readings were obtained at 50 cm from the ground level, close to the carrot crop canopy.

In both experimental sites, air temperature and relative humidity were measured with a thermistor probe Model HMP 35 (Campbell Scientific Inc., Logan, UT, USA). The soil temperature was measured with a thermistor sensor embedded into a plastic waterproof probe, Model 107B (Campbell Scientific Inc., Logan, UT, USA). Soil water content was measured by the gravimetric method. The rainfall was measured with a tipping-bucket rain gauge (Campbell Scientific Inc., Logan, UT, USA). The leaf wetness was measured by a Flat Leaf Wetness Sensor, Model 237 (Campbell Scientific Inc., Logan, UT, USA). In each experimental site there were two Flat Leaf Wetness Sensors, one exposed to the external atmospheric factors and the other under the canopy to receive canopy micro-climate readings. All sensors were attached to a CR 21X data logger (Campbell Scientific. Inc., Logan, UT, USA) established in the middle of the plot in both experimental sites.

B) Monitoring pathogen's variables

In both experimental plots, a diamond shaped pattern was used to obtain a total of 8 sample sites for conducting all monitoring. Each sample site was represented by a 2 m long and 0.86 m (Site 1) or 0.72 m (Site 2) wide section of carrot row (covering half of both sides of two carrot hills).

i) Weekly observations for germination of apothecia started 26 July 1999, in both experimental sites. Germinated apothecia were counted as total number present in all sample sites of each experimental plot on the day the observation was conducted, and apothecia per unit square metre were estimated.

ii) Weekly observations for airborne ascospores commenced 26 July 1999, in both experimental sites. The presence and concentration of viable ascospore deposition over time were monitored and measured using the Blue Plate Technique (Steadman & O'Keefe, 1998). In each sample site, one open petri dish of 90 mm of diameter, containing about 15 ml of Sclerotinia Selective Medium (SSM- consisting of 39 g Potato Dextrose Agar (PDA), 25 g Pentachloronitrobenzene (PCNB), 150 g Penicillin, 150 g Streptomycin sulphate and 50 g Bromophenol blue per 1 liter of distilled water), was placed on the ground under the canopy, in an angled position facing south-west to act as a spore trap. On every observation, the plates were exposed in the field for three consecutive hours, from 10:00 am - 13:00 pm. This is reported as the optimum time for the maximum release of the ascospores from the apothecia (Steadman & O'Keefe, 1998). The plates were then covered and brought to the lab where they were incubated at 20°C for 3-5 days. The colony forming units (CFU) of ascospores were counted for each plate (based on the colour change of the medium from blue to yellow, due to the oxalic acid released by *S. sclerotiorum*) and mean per unit square centimetre of media plate for each experimental plot was estimated.

C) Monitoring disease incidence

Pre and post-symptomatic assessments were performed starting 26 July 1999, by biweekly destructive sampling of two full carrot plants, randomly selected and collected from each sampling site. Randomly selected sub-samples of leaflets, petiole sections and carrot root slices from each plant, were surface sterilized (30 seconds in 75% Ethanol, 1 minute in 10% bleach solution, 3 minutes in sterile water), plated on SSM, and incubated for 7-14 days at 20-21°C. The leaflet and petiole units were taken separately from young and healthy leaves in upright position, from senescent leaves in upright position, and from senescent leaves laying on the ground, still attached to the plant. The units that tested positive for sclerotinia rot infection (confirmed by the change of the media colour and sclerotia formation) were counted, and percent disease incidence was calculated for the total number of units observed.

D) Monitoring survival of sclerotia buried in muck soil over time

Nylon mesh sacs (5x5 cm) containing 30 sclerotia of *S. sclerotiorum* each, were buried 5 cm deep into contained areas of muck soil field at Muck Crop Research Station (MCRS) in November 1998. The 2 year old sclerotia, collected from a soybean seed lot, were allowed to overwinter in the field for a period of 5 months and were recovered the following spring, when the soil was fully thawed. The recovery was conducted in one month intervals over a period of 5 months, starting 15 April until 15 September 1999. At each recovery, the sclerotia that were

physically intact were surface sterilized (1 minute in 75% Ethanol, 3 minutes in 10% bleach solution, 3 minutes in sterile water), blot dries and plated onto SSM. They were incubated for 7-10 days at 20-21^{0}C. All germinated sclerotia were counted as viable and percent viability was estimated for the total number of buried sclerotia.

2. Potential integration of control methods in onion and carrot production

3. a Assessment of the potential integration of DADS applications with the cut-and-peel carrots cropping system and DADS residue determination on carrots roots

Carrots were direct seeded (96 seeds/m) into soil which had been treated with DADS prior to seeding on 15 June using a StanHay precision seeder. Samples of 25-30 carrots were harvested on 24 Aug. These carrots were sent to Labs Services Division for DADS analysis. The remaining carrots were grown to maturity and harvested on 28 Oct. Carrots were then stored in a Filacell storage at 1^{0}C and 90% humidity until 7 March when the taste testing took place. The carrot samples included were: the carrots grown in the DADS treated soil, Cellobunch a standard packaging carrot cultivar, a Nantes type carrot, which is known for sweet flavour, and Fontana, a standard processing cultivar. The untreated check from the plot was also included in the taste test. The evaluation panel consisted of five people. Before sampling took place five carrots of each cultivar were washed and 0.5-0.8 cm discs were cut from the middle area of the carrot. Each carrot sample was evaluated for harsh biting flavour, sweetness, overall carrot flavour and general preference. Carrots were also tested for sugar content using a hand held Refractometer. Data were analyzed using the General Analysis of Variance function of the Linear Models section of Statistix V.4.1.

3.b Evaluation of the effects of white rot bio-control *T. harzianum* on survival of sclerotia of *S. sclerotiorum* and the effect of *C. minitans* and *Sporodegmium sclerotivorum* on the survival of sclerotia of *S. cepivorum* (Field and greenhouse trials).

These aspects were covered in sections 1 and 2 due to the unavailability of the commercial formulation of *C. minitans* and *Sporodegmium sclerotivorum* after 1998.

WEATHER CONDITIONS

1997

Air temperatures were below the long term (10 year) average for May, Jul and Aug, above for Jun and not different from the long term average for Sept and Oct. Total rainfall was below the long term (10 year) average for May (62.2mm), Jun (65.8mm), Jul (25.6mm), Aug (48mm) and Oct (32mm) and above for Sept (119mm). The lack of accumulated precipitation and the lack of irrigation resulted in a drought stress situation.

1998

Air temperatures were above the long term (10 year) average for May, and not different from the long term average for Jun, Jul, Aug and Sept. Total rainfall was below the long term (10 year) average for May (42.6 mm), Jul (50.2 mm) and Sept (18.6 mm); above average for Aug (114.6 mm) and not different from the long term average for Jun (78.4 mm). The lack of accumulated precipitation and the lack of irrigation resulted in a drought stress situation at all sites.

1999

Air temperatures were above the long term (10 year) average for Jun, Jul and Sept, and below average for Aug. Total rainfall was below the long term (10 year) average for Jun (68.5 mm), Jul (71 mm) and Aug (78.8 mm) and above average for Sept (137.5 mm). The lack of accumulated precipitation and the lack of irrigation resulted in a drought stress situation.

RESULTS AND DISCUSSION

1. Management of onion white rot

1.a Evaluation of biological agent *Trichoderma harzianum* for the control of onion white rot

Treatments with two formulations of *Trichoderma harzianum* were not effective in reducing white rot over the 3 years they were tested. Incidence of white rot was relatively high in 1997, low in 1998 and moderate in 1999. No significant differences were found among treatments or within the cultivars tested (Table 1.a.1.1.).

Under the test conditions, *Trichoderma harzianum* did not appear to control onion white rot. Throughout the growing season the rhizosphere, the area around the plant root, is subject to rapid and long-term fluctuations in water, salt, pH, nutrients, temperature and microorganism populations due to plant growth and development and changing environmental and climatic conditions. It is not understood what these changes are or how the changes affect the efficacy of *T. harzianum* in controlling white rot. *T. harzianum* has a broad range of activity against several plant pathogens utilizing various control mechanisms. With many biocontrol agents, activity can vary from year to year and site to site.

Table 1.a.1.1. Incidence of white rot (%) in onion cultivars treated with granular and drench formulations of *Trichoderma harzianum*, grown at commercial sites in the Holland Marsh, Ontario, in 1997 - 1999.

			Treatments				
Year	**Site**	**Cv.**	**Check**	**T-22 RSD**	**T-22RSD**	**T-22G**	**T-22G**
1997		Rate /100 m of row ->	--------	2.34 g	4.67 g	46.7 g	NA*
	Site 1	Fortress	0 NS**	0	0	0	NA
		Hustler	0 NS	0	0	0.24	NA
	Site2	Hustler	22.27 NS	20.6	34.46	45.43	NA
1998		Rate / 100 m of row ->	--------	4.48 g	22.5 g	44.8 g	NA
	Site 1	Fortress	0.58 NS	1.17	0	0	NA
		Frontier	0 NS	0	1.06	0	NA
	Site 2	Fortress	3.60 NS	0	3.85	1.82	NA
		Frontier	1.11 NS	0.3	0.43	0	NA
1999		Rate / 100 m of row ->	--------	40 g	80 g	120 g	240 g
	Site 1	Fortress	6.67 NS	5.09	3.3	5.56	6.86
	Site 2	Fortress	12.69 NS	11.63	5.53	6.92	17.99

* NA - not applicable

** NS - no significant treatment effects were observed (P=0.05)

1.b Field assessment of the efficacy of DADS for reducing onion white rot

Incidence of white rot was relatively low in 1997, 1998 and 1999 (maximum 13.02, 3.66 and 6.89 % respectively), but treatment differences were identified. Single treatments with DADS significantly (P=0.05) reduced white rot on onions compared to the untreated check at Site 3 (81%) while two applications of DADS significantly reduced white rot by 99, 100, 86 and 96% (sites 1, 2, 4 and 5 respectively). Single treatments with DPDS did not reduce white rot on onions compared to the untreated check while two applications of DPDS significantly reduced white rot by 46% compared to the untreated check at site 1 only (Table 1.b.1.1.).

To maximize the effect of DADS and DPDS on the natural populations of sclerotia found in the field sites, soil moisture was monitored pre- and post-treatment. At no time was there insufficient moisture for germination of sclerotia and product diffusion through the soil complex.

Treatments were applied late summer or early fall to ensure that the soil temperatures would remain between 9 and 20°C for two to three months, optimal for sclerotia germination and product diffusion. The soil structure was well worked pre-treatment and rolled to create a seal post-treatment. No white rot susceptible crop was re-planted in the treated area until the spring following one or two soil treatments. Field incidence of white rot was relatively low (0 to 13.7%) at all sites (1997 and 1998) due to the hot, dry weather during the growing season, which resulted in moderate white rot development. In cool, wet growing seasons, the incidence of white rot in commercial fields can exceed 65 percent. Nevertheless, in 4 out of 5 field sites, white rot incidence at harvest was reduced significantly compared to the check by 72 to 88 percent using one application of DADS and 86 to 100 percent using two applications at 10L/ha in 500L/ha of water, in consecutive years.

The number of viable *S. cepivorum* sclerotia/g of soil in most heavily infested commercial fields in the Holland Marsh ranges from 0.1 to 0.3 (unpublished data). Tichelaar (1965) found that populations of 0.002 to 0.03 sclerotia/g of mineral soil resulted in 25 to 40 percent infection. In muck soil, in the Fraser Valley, populations of 0.07 to 0.15 sclerotia/g resulted in 5.9 to 21.6 percent infection (Leggett and Rahe 1985).

Table 1.b.1.1. Evaluation of DADS, DPDS and untreated check for the control of white rot in muck soils, in the Holland Marsh, Ontario.

Site	Treatments	Treatment date	Assessment date	% white rot incidence
1	DADS (10 L/ha DPDS (10 L/ha) Untreated check	Aug'95 & Sept'96	Sept '97	0.07a* 7.05b 13.02b
2	DADS (10 L/ha) DPDS (10 L/ha) Untreated check	Aug'95 & Sept'96	Sept '97	0.0a 1.51a 5.19b
3	DADS (10 L/ha) Untreated check	Sept'96	Sept '98	0.71a 3.66b
4	DADS (10 L/ha) Untreated check	Sept'96 & Sept'97	Sept '98	0.13a 0.94b
5	DADS (10 L/ha) Untreated check	Sept'96 & Sept'97	Sept '98	0.09a 2.35b

* Numbers in a column (by site) followed by the same letter are not significantly different at P=0.05, Fisher's Protected LSD Test.

1.c Survival of sclerotia of *Sclerotium cepivorum* exposed to DADS

In a controlled greenhouse study, there were no significant (P=0.05) differences in total mortality of sclerotia between the DADS, DPDS and the check treatment after 1 month of exposure of sclerotia to the treated soil. Exposure to DADS resulted in significantly higher sclerotia mortality compared to DPDS and the untreated check after 2 and 3 months exposure. The assessments of surviving sclerotia from trials in 1999 are still in progress (Table 1.c.1.1.)

Table 1.c.1.1. Effect of DADS and DPDS on the survival of white rot sclerotia in a greenhouse pot trial, comparison between treatments within each assessment.

	Germination of sclerotia (%)		
Treatment	**1st month**	**2nd month**	**3rd month**
DADS	78.00a	15.35a	18.35a
DPDS	82.00a	88.00b	60.05b
Check	100.00a	99.18c	99.18c

* Numbers in a column followed by the same letter are not significantly different at P=0.05, Fisher's Protected LSD Test.

The effect of exposure to DADS on the mortality of sclerotia was examined in four field sites. The site by treatment interaction was not significant at (P=0.05) for field loss , loss + lab and total mortality after 3 and 7 months exposure to the germination stimulants. When sclerotia were exposed for 3 months to the soil treatments, significant differences between treatments for total (P=0.031) mortality were found. There was no site effect but treatment was significant (P=0.034). DADS resulted in a higher percent loss of sclerotia (lower recovery, 84.21%) than the untreated check (74.90%) (Table 1.c.1.2).

Table 1.c.1.2. Effect of DADS on the survival of white rot sclerotia in a field trials, no site 1.

	Field loss		Total mortality	
Site	Fall(3)*	Spring(7)	Fall(3)	Spring(7)
2	76.92	74	90.3	83.81c
3	76.29	73.79	90.86	87.79bc
4	80.08	83.67	99.81	92.74ab
5	84.92	79.67	92.62	98.60a
Treatment				
DADS	84.21a	79.22	96.58a	90.85
Control	74.90b	73.75	90.22b	90.61

* Time after exposure to DADS (3 or 7 months).

** Numbers in a column followed by the same letter are not significantly different at P=0.05, Fisher's Protected LSD Test.

In the greenhouse and field studies, two sclerotia germination stimulants were utilized at rates recommended by the supplier and potential product registrant (United Agri Products). To avoid potential sources of interference with product efficacy, sclerotia were at least four months old prior to use so that constitutive dormancy was not a factor (Coley-Smith *et al* 1987), and had greater than 90 percent germination potential. Only one product, DADS, resulted in consistent and significant reductions in populations of viable sclerotia based on assessments of sacs containing a pre-determined number of sclerotia and exposed to the treatments. In the controlled greenhouse study, DADS resulted in an average of 85 percent sclerotia mortality compared to less than one percent in the untreated check. Crowe *et al* (1991) also found that sclerotia in untreated areas died as a result of stimulated germination. They found that the volatilized treatment product was able to diffuse through the mineral soil into the untreated areas but did not go beyond three meters as determined by assessing sclerotia viability. Our field trial areas ranged from 2.43 to 10.97 meters wide and were separated by 0.3 meters. Therefore, it is possible that some of the DADS was able to stimulate sclerotia buried in the untreated areas.

In the field, total loss was over 95 percent after three months exposure to the treated soil while the control was less than 90. Even though the total loss in the field trials after 7 months exposure to DADS was over 90 percent there were no significant differences between treatments (Table 1.c.1.2). One month exposure to DADS was not sufficient time to decrease the sclerotia viability significantly in the greenhouse (Table 1.c.1.1.). This result agrees with work done by

Sommerville and Hall (1987), that germination of sclerotia when exposed to allyl products was a linear response with time, increasing from 15 percent (at 15 days) to over 55 percent (at 45 days). Crowe *et al* (1991) reported that the residual capacity of stimulant-treated mineral soil was high for a month after application, but declined by 6 to 8 weeks.

The loss of sclerotia in the field check plots was high after 3 and 7 months and the lack of an increased response in sclerotial loss in the DADS treatments after 7 months was likely a direct result of natural survival of sclerotia, soil temperature and soil moisture. Leggett and Rahe (1985) found that sclerotia of *S. cepivorum* buried in muck soil decayed with time and the rate of decay was influenced by environmental conditions that appeared to be highest during the winter months when soil tended to be water logged and temperatures were less than 10°C. Mean soil temperature, after Nov, in the Holland Marsh, Ontario, until spring is low enough to decrease the survival rate of the sclerotia. Germination of sclerotia is also inhibited when soil temperatures are below 10°C (Crowe and Hall 1980) and volatile products cannot diffuse as well through soil complexes. Therefore, to decrease populations of natural sclerotia under field conditions using DADS, the soil should be at 50 percent moisture for sclerotia germination and product diffusion, temperatures should be between 9 and 20°C for two to three months and the soil complex should be well worked to enhance product diffusion.

1.f Evaluation of onion breeding lines for resistance to white rot

A) Scale inoculation

Significant (P=0.05) differences were found in lesion diameters among onion lines and cultivars. There was a significant MCG isolate by onion interaction in 1998 and 1999, there was not a significant MCG isolate by onion interaction in 1997, the results from the two isolates were pooled (Table 1.f.1.1).

Within MCG-1 and MCG-2, the breeding lines from the University of Wisconsin had the largest lesions, and the smallest lesions were found on the Petoseed lines (in contrast to previous years), but the contrast was not significant. The University of Wisconsin breeding line, W91297 had the largest lesion diameter which was significantly larger than most of the other breeding lines and cultivars when inoculated with MCG-1 and MCG-2 (Table 1.f.1.2).

The Petoseed, PS650096 breeding line had the smallest lesion diameter when inoculated with all 3 MCG's and was significantly smaller than approximately one third of the other breeding lines and cultivars (Table 1.f.1.1). There was a highly significant, positive Pearson correlation among

diameters of lesions formed by MCG-1 and MCG-2 on the 18 breeding lines (R^2=0.61, P=0.00001) (Data not shown).

The onion lines which had the largest lesion diameters when inoculated with MCG-1, also had the largest lesion diameters when inoculated with MCG-2 (Table 1.f.1.3). The resulting correlation is not high, indicating that there are more factors than the onion cultivar/breeding line and MCG involved in determining the resulting lesion diameter. MCG-1 tended to result in larger lesions overall than MCG-2; the isolate, MCG-1 is considered more virulent than MCG-2. The MCG-3 cultures used for inoculation resulted in highly variable results among replicates with no significant differences among cultivars. MCG-3 was consistently less pathogenic than MCG-1 and MCG-2.

There was a significant (P=0.0001) MCG isolate by onion cultivar interaction, therefore, the results from the three isolates could not be pooled. Significant (P=0.05) differences in lesion diameters (20.5 to 31.8 mm) were found among onion cultivars harvested in 1997 and inoculated with MCG-2 (Table 1.f.1.1). There was no significant (at P=0.05) Pearson correlation between lesion diameters formed by MCG-1 and MCG-2. There were no significant differences in lesion diameters among cultivars when inoculated with either MCG-1 or MCG-3. It was observed that the MCG-1 stock cultures appeared to have slower mycelial growth than previously seen. The MCG-3 cultures used for inoculation resulted in highly variable results among replicates with no significant differences among cultivars. MCG-3 was consistently less pathogenic than MCG-1 & 2.

Table 1.f.1.1. Lesion diameters on onion scales inoculated with *Sclerotium cepivorum* mycelia,1997.

Onion	Lesion diameters (mm)		
	MCG pooled	MCG-1 1-9	MCG-2 3-6
PSR459194*	25.38 a***	28.75 a	22.00 ab
PSR459294	24.81 ab	29.38 a	20.25 a-f
PSR459694	23.50 a-c	27.50 ab	19.50 a-g
WR458	23.25 a-d	24.63 a-e	21.88 a-c
FORTRESS	23.06 a-d	27.50 ab	18.63 a-h
JOINT VENTURE	23.06 a-d	24.38 a-e	21.75 a-d
PSR459094	22.31 a-e	25.00 a-d	19.63 a-g
W455B**	22.19 a-f	26.25 a-c	18.13 a-h
XW455B	22.06 a-f	24.88 a-e	19.25 a-g
W454B	21,63 a-g	20.38 c-f	22.88 a
PSR459394	20.81 b-h	23.38 a-e	18.25 a-h
XW459C	20.75 b-h	23.38 a-e	18.13 a-h
XW458C	20.44 c-h	23.75 a-e	17.13 c-h
PSR459494	20.25 c-h	22.63 b-f	17.88 b-h
PSR459594	20.13 c-h	24.25 a-e	16.00 f-h
NORSTAR	20.06 c-h	18.88 ef	21.25 a-e
WR459	19.69 c-h	22.13 b-f	17.25 b-h
W459C	19.13 d-h	21.50 b-f	16.75 e-h
W458C	19.13 d-h	21.25 c-f	17.00 d-h
W456C	18.81 e-h	20.00 d-f	17.63 b-h
XW456C	18.50 e-h	22.00 b-f	15.00 gh
W457C	18.00 f-h	19.13 d-f	16.88 eh
PSR458994	17.50 gh	21.13 c-f	13.88 h
PARAGON	16.75 h	17.25 f	16.25 f-h

* Lines beginning with the designation "PSR" and "WR" were breeding lines from Petoseed.

** Lines beginning with the designation "W" and "XW" were breeding lines from the University of Wisconsin.

*** Numbers in a column followed by the same letter are not significantly different at P=0.05, Fisher's Protected LSD Test.

Table1.f.1.2. Onion scale lesion diameters 7 days after *Sclerotium cepivorum* mycelia inoculation, 1998.

Onion Cultivar/Line	Source	Lesion diameters (mm)		
		MCG-1	MCG-2	MCG-3
W91297	Wisconsin	32.6 a*	30.7 a	21.5
W91097	Wisconsin	32.1 a	26.4 b	19.3
Norstar	Stokes	29.6 ab	26.8 b	16.3
W10596	Wisconsin	28.8 bc	26.6 b	18.2
PSW457	Petoseed	28.7 bc	24.7 bc	17.8
W10196	Wisconsin	28.7 bc	24.3 b-d	17.5
Paragon	Sun Seeds	28.2 b-	26.2 b	14.6
XPH15055	Asgrow	28.1 b-e	24.8 bc	17.2
W10496	Wisconsin	28.0 b-e	23.8 b-e	17.6
W92097	Wisconsin	27.5 b-f	24.7 bc	16.2
Joint Venture	Stokes	27.2 b-f	21.5 c-e	17.7
Fortress	Asgrow	27.2 b-f	26.8 b	16.8
W91897	Wisconsin	27.1 b-f	23.4 b-e	15.7
W91697	Wisconsin	27.1 b-f	24.0 b-e	18.9
Prince	Seedway	26.7 b-	24.9 bc	14.3
Hamlet	Asgrow	26.2 c-h	24.1 b-e	18.9
PSWR465	Petoseed	26.1 c-h	20.9 de	16.1
W10296	Wisconsin	25.7 d-h	23.5 b-e	17.8
PS650196	Petoseed	25.2 e-h	24.5 b-d	18.2
W91497	Wisconsin	25.2 e-h	24.1 b-e	15.1
PS650396	Petoseed	25.0 f-h	23.3 b-e	17.2
W92497	Wisconsin	24.6 f-h	21.8 c-e	15.9
PS650296	Petoseed	24.1 gh	20.9 de	15.5
PS650096	Petoseed	23.5 h	20.4 e	13.9

* Numbers in a column followed by the same letter are not significantly different at P=0.05, Fisher's Protected LSD test.

** NS - no significant treatment effects were observed.

Table 1.f.1.3. Onion scale lesion diameters 7 days after *Sclerotium cepivorum* mycelia inoculation, 1999.

		Lesion diameters (mm)	
Onion Cultivar/line	**Source**	**MCG-1**	**MCG-2**
Corona	Bejo/Seedway	38.563 a*	32.5
W454B	Wisconsin	31.313 b	26.1
Frontier	American Takii	31.250 b	35.6
WR457	Petoseed	30.375 bc	25.8
Norstar	Stokes	29.750 bd	23
XPH15055	Asgrow	29.688 bd	27
W429AxW4	Wisconsin	27.938 be	24
W455B	Wisconsin	27.750 bf	28.6
W457C	Wisconsin	27.563 bf	24.6
Prince	Stokes	27.063 bf	25.5
WR447	Petoseed	26.438 cg	22.3
Fortress	Asgrow	25.938 cg	28
B9011	Wisconsin	25.375 ch	23.3
PSR650396	Petoseed	24.438 ei	24.7
W459C	Wisconsin	24.313 ei	24.7
PSR650196	Petoseed	24.000 ei	20.5
Turbo	Stokes	23.813 ei	25
PSR650296	Petoseed	23.813 ei	21.4
W440AxW4	Wisconsin	23.688 ei	23
TopNotch	Stokes	23.688 ei	25.5
W458C	Wisconsin	23.563 ei	21.3
Headliner	Petoseed	23.500 ei	19.5
Joint Venture	Stokes	23.188 fi	22.6
W434AxW4	Wisconsin	22.250 gi	22.3
WR1752	Petoseed	22.188 gi	18.7
W460C	Wisconsin	22.000 gi	24.3
W456C	Wisconsin	21.938 gi	22.6
PSR650096	Petoseed	21.063 hi	18.8
WR456	Petoseed	20.813 hi	23.2
W461B	Wisconsin	20.750 hi	23.5
XPH15056	Asgrow	20.063 i	23.6

* Numbers in a column followed by the same letter are not significantly different at P=0.05, Fisher's protected LSD Test.

B) Field trials

Incidence of white rot was low at all sites in 1997. Site 1 did not develop any signs of infection by *Sclerotium cepivorum* at harvest. White rot incidence was not significant (P=0.05) within cultivars and breeding lines at sites 3 and 4. Significant differences among cultivars and breeding lines were found at site 2. The cultivar Fortress did not develop symptoms of white rot infection and had significantly less white rot than Hamlet, Joint Venture, W101-96, Prince, W102-96 and P5650396. When the breeding lines were grouped, the contrast between the University of Wisconsin and Petoseed breeding lines was not significant (P=0.220). The breeding material for the resistant lines for both the University of Wisconsin and Petoseed came from original work and material from concurrent work done between Dr. J. Rahe at Simon Fraser University in British Colwnbia (Rahe, 1986) and Dr. W.H. Gabelman at the University of Wisconsin (Gabelman, 1986). Therefore, the genetic material is similar and it was not surprising that the contrast between the two groups was not significant (Table 1.f.1.4.).

In 1998 and 1999 incidence of white rot incidence ranged from 0 to 12.8% at the commercial field site and in the pot trial. Significant differences were found among cultivars tested in the field trial. Three cultivars had the highest incidences of white rot while almost no significant differences were found among the breeding lines (Table 1.f.1.5. and Table 1.f.1.6.).

Table 1.f.1.4. Harvest incidence of white rot in onion lines grown at three sites in the Holland Marsh, Ontario, in 1997.

Onion line	White rot incidence (%)		
	Site 2	Site 3	Site 4
HAMLET	3.76 a****	2.57 a	0
JOINT VENTURE	3.60 ab	0.32 a	0.33 a
W101-96*	3.59 ab	1.04 a	0
PRINCE	2.92 a-c	1.89 a	0.31 a
W102-96	2.58 a-d	-----	0
PS650396**	2.54 a-d	0.68 a	0.68 a
PS650196	2.42 a-e	-----	0.28 a
W912-97	2.29 a-e	-----	0
PS650096	2.24 a-e	-----	0.31 a
PSW457	2.05 a-e	-----	0
W916-97	1.92 a-e	-----	0
XPH15055***	1.41 a-e	1.09 a	0
PARAGON	1.29 a-e	0.99 a	0.61 a
W104-96	1.27 b-e	-----	1.07 a
W918-97	0.97 c-e	-----	0
W914-97	0.68 c-e	0.32 a	0
W920-97	0.66 c-e	1.25 E-17 a	0.64 a
PSWR456	0.66 c-e	0 a	1.30 a
W105-96	0.63 c-e	-----	0.30 a
W924-97	0.37 de	0	1.49 a
PS650296	0.32 de	0.30 a	0.37 a
NORSTAR	0.31 de	0.32 a	0.33 a
W910-97	0.30 de	-----	0
FORTRESS	0 e	5.78 E-18 a	0 a

* Lines beginning with the designation "W" were breeding lines from the University of Wisconsin

** Lines beginning with the designation "P" were breeding lines from Petoseed.

*** The line beginning with the designation XPH" was a breeding line from Asgrow Ltd.

**** Numbers in a column followed by the same letter are not significantly different at P=0.05, Fisher's Protected LSD Test.

Table1.f.1.5. White rot harvest incidence in onions at one commercial field site and in a pot trial, 1998.

		White rot incidence (%)	
Cultivar/Line	**Source**	**field site**	**Pot**
Turbo	Stokes	12.8 a*	0 NS**
Top Notch	Stokes	6.9 b	4.2
Headliner	Petoseed	3.7 c	0
PSR650296	Petoseed	2.7 cd	3.6
PSR650096	Petoseed	2.5 c-e	0
Joint Venture	Stokes	2.3 c-e	0
Prince	Bejo/Seedway	1.8 c-e	6.7
PSR650196	Petoseed	1.3 c-e	5
WR1752	Petoseed	1.3 c-e	10.7
XPH15055	Asgrow	1.1 de	3.6
Norstar	Stokes	0.9 de	0
Corona	Bejo/Seedway	0.8 de	0
104-96	Wisconsin	0.7 de	0
918-97	Wisconsin	0.7 de	7.1
PSR650396	Petoseed	0.7 de	10.7
Frontier	American Takii	0.7 de	0
914-97	Wisconsin	0.5 de	3.6
906-98	Wisconsin	0.4 de	3.6
101-96	Wisconsin	0.4 de	4.2
WR447	Petoseed	0.2 de	5
920-97	Wisconsin	0.2 de	0
XPH15056	Asgrow	0.2 de	10.7
912-97	Wisconsin	0.2 de	0
WR457	Petoseed	0.2 de	3.6
B901-1	Sun Seeds	0.2 de	0
916-97	Wisconsin	0.2 de	8.3
102-96	Wisconsin	0.2 de	0
Fortress	Asgrow	0.2 de	3.6
910-97	Wisconsin	0 e	3.6
924-97	Wisconsin	0 e	0
WR456	Petoseed	0 e	5

* Numbers in a column followed by the same letter are not significantly different at P=0.05, Fisher's Protected LSD Test. ** NS - no significant treatment effects were observed.

Table 1.f.1.6. White rot harvest incidence in onions at two commercial field site and in a pot trial, 1999.

		White rot incidence (%)		
Cultivar /Line	**Source**	**Site 1**	**Site 2**	**Pot trial**
Benchmark	Asgrow	3.48 a	4.14 a-d	7.40 a
Hoopla	J.C. Canners	2.44 ab	4.58 ab	0 c
Hamlet	Asgrow	2.38 a-c	4.05 a-d	0 c
W462C	U of Wisconsin	1.64 a-d	2.39 b-e	0 c
PSFemale6	Petoseed	1.59 a-d	0.81 b-e	NA
Top Notch	Stokes	1.55 b-d	1.08 b-e	0 c
1220B	Asgrow	1.36 b-d	0.72 c-e	NA
1596B	Asgrow	1.14 b-d	0.69 c-e	NA
Norstar	Stokes	1.12 b-d	1.20 b-e	0 c
1247B	Asgrow	0.71 b-d	0 e	NA
W460C	U of Wisconsin	0.71 b-d	0.30 de	0 c
PSFemale5	Petoseed	0.50 cd	0 e	NA
W461B	U of Wisconsin	0.42 d	0.77 b-e	0 c
Joint Venture	Stokes	0.40 d	1.74 b-e	0 c
Turbo	Stokes	0.36 d	0.36 de	3.70 b
1415C	Asgrow	0.36 d	1.41 b-e	NA
XPH15055	Asgrow	0.36 d	2.63 b-e	NA
PS650196	Petoseed	0.35 d	4.37 a-c	0 c
Fortress	Asgrow	0.35 d	1.41 b-e	0 c
PSW456	Petoseed	0.35 d	0.65 c-e	NA
Prince	Bejo/Seedway	0.34 d	6.89 a	0 c
1231B	Asgrow	0.34 d	0.63 c-e	NA
W463B	U of Wisconsin	0.34 d	0.34 de	0 c
XPH15056	Asgrow	0.33 d	1.99 b-e	NA
PSW457	Petoseed	0 d	0.98 b-e	NA
B901-1	Sun Seeds	0 d	1.73 b-e	NA
PS650096	Petoseed	0 d	0.76 b-e	0 c
Frontier	American Takii	0 d	0 e	0 c
Headliner	Petoseed	0 d	2.31 b-e	0 c
Corona	Bejo/Seddway	0 d	0 e	0 c
1416C	Asgrow	0 d	0.42 de	NA
PS650396	Petoseed	0 d	0.70 c-e	NA
PS650296	Petoseed	0 d	2.30 b-e	0 c
Millenium	Sun Seeds	NA**	2.65 b-e	NA

* NS- not significant differences were observed at P=0.05, Fisher's Protected LSD test.
** NA - not applicable

B) Management of Sclerotinia Rot of Carrot

2.a Assessment of the efficacy of field applied fungicides and calcium nitrate, Nitro-Plus, calcium propionate, sodium bicarbonate, and sodium hypochlorite washes as post-harvest dips on development of sclerotinia rot in cut-and-peel carrots

In 1998 significant differences were found among treatments on both assessment dates. Sclerotinia rot infection (%) was the lowest in Jan and May in the Bravo 500 drench and the unwashed field check, but they were not significantly different from any of the other fungicide treatments. The five fungicide treatments had significantly lower percent infection than the Calcimax 0.1% drench and the washed field and washed drench checks in January and May. Both calcium treatments had high levels of infection but were significantly lower than the washed drench check in May. The washed field and drench checks had the highest percent infection both in January and May.

Table 2.a.1. Evaluation of various field and post harvest fungicide and calcium treatments for the control of sclerotinia rot on carrots in storage, 1997-98.

Treatments	Rate of Product	Disease Infection (%)	
		Jan 98	May 98
Unwashed Field Check		0.3 a *	4.7 ab
Washed Field Check		14.8 e	23.3 cd
Washed Drench Check		13.3 de	25.1 e
BENLATE 50 WP	1.1 kg/ha	2.9 ab	7.7 ab
BRAVO500	3.2 L/ha	4.1 ab	5.5 ab
BENLATE 50 WP Drench	2.2 g/L	2.1 ab	9.6 ab
BRAVO 500 Drench	6.0 mL/L	0.8 a	1.5 a
BOTRAN 75 W Drench	3.7 g/L	1.4 ab	9.2 ab
CALCIMAX 0.1% Drench	1.25 mL/L	9.4 cd	20.3 cd
NITRO 9 1.0% Drench	1.42 mL/L	5.9 b	13.9 bc

* Numbers in a column followed by the same letter are not significantly different at P = 0.05, Fisher's Protected LSD Test.

In 1999 significant differences were found among treatments on both assessment dates. The Ronalin spray treatment resulted in significantly lower percent infection than the washed check but not the field check in February. All post harvest drench treatments had a higher percent infection than the field check. The Nitro 9 drench at 14.3 ml/L and Pathoclean had significantly

more diseased carrots than the washed check in February. Pathoclean was the only treatment with significantly higher damage than both the field and the washed check in July. Nova Cal 2000 kg/ha had the lowest percent infection in July at 14.3%. The Benlate 50 WP at 1.1 kg/ha in 1000 L/ha, CM spray and the Calcimax spray treatment also had infections under 20%. No significant differences were observed in the degree of infection in March, however the Pathoclean treatment had a rating of 3.3, almost one point lower than any other treatment. Significant differences were found in July in the degree of infection. Pathoclean had the lowest degree rating and was significantly different from all treatments except the Nitro 9 drench at 1.4 ml/L.

Table 2.a.2. Evaluation of various field and post harvest fungicide and calcium treatments for the control of Sclerotinia Rot on carrots in storage, 1998-99.

Treatment	Rate of Product	Disease Infection (%)		Degree of Infection*	
		March	July	March	July
Field Check	-	0.8 a**	22.6 ab	4.8 a	4.3 a-d
Washed Drench Check	-	14.2 cd	37.0 abc	4.3 a	3.0 efg
Benlate 50WP	1.1 kg/ha in 500 L	4.6 ab	44.1 bc	4.3 a	3.0 efg
Benlate 50WP	1.1 kg/ha in 1000 L	0.5 a	18.6 ab	4.6 a	4.3 a-d
Ronalin	2.25 kg/ha	0.3 a	20.2 ab	4.8 a	4.2 a-e
Nitro 9 Spray	5% solution	1.6 a	29.8 abc	4.5 a	3.7 a-f
Calcimax Spray	5% solution	1.7 a	16.2 a	4.4 a	4.4 abc
Nova Cal	1000 kg/ha	2.8 a	31.9 abc	4.2 a	3.5 b-g
Nova Cal	2000 kg/ha	0.5 a	14.3 a	4.6 a	4.7 a
CM - Spray	1.25×10^{13} spores/ha	0.2 a	17.4 ab	4.8 a	4.5 ab
CM - Field Drench	1.25×10^{13} spores/ha	0.3 a	30.8 abc	4.3 a	4.4 abc
Benlate Drench	2.2 g/L	7.4 abc	31.6 abc	4.7 a	3.2 d-g
Calcimax Drench	12.5 ml/L	17.3 de	34.2 abc	4.4 a	3.3 c-g
Nitro 9 Drench	1.42 ml/L	22.5 e	55.9 cd	4.3 a	2.4 gh
Nitro 9 Drench	14.3 ml/L	10.6 bcd	20.4 ab	4.7 a	3.4 b-g
Pathoclean	2.0 ml/L	40.9 f	74.6 d	3.3 a	1.5 h
Hydro-Cooling	4^0C for 2 min.	15.0 cde	38.9 abc	4.2 a	2.9 fg

* 5.0 = No Disease, 3.7 = Moderate, 1.0 Severe (liquefied)

** Numbers in a column followed by the same letter are not significantly different at P = 0.05, Fisher's Protected LSD Test.

2.b Laboratory assessment of the efficacy of calcium nitrate, Nitro-Plus, calcium propionate, sodium bicarbonate, and sodium hypochlorite washes as post-harvest dips on development of sclerotinia rot in cut-and-peel carrots

In the cold storage experiments in both 1998 and 1999, incubation at 1□C inhibited completely growth and decay by *S. sclerotiorum* over the 8 week cold-storage period (data not shown). However, incubations at 6 °C were complicated by malfunctions in the cold storage equipment resulting in periodic temperature elevations to as much as 10-12 °C for 1 to 2 day periods. During this time, the Sclerotinia rot and growth of the cottony mycelium on inoculated carrots (length of lesions) and spread from carrot to carrot (width of lesions) was enhanced greatly. None of the chemical washes resulted in a significant (P=0.05) suppression of white rot and spread of *Sclerotinia* mycelium (Fig. 3). Significant inhibition by calcium propionate washes was observed in the 1999 experiments. There was a trend towards inhibition by washes with sodium hypochlorite and calcium propionate in 1998 tests. Phytotoxicity and enhanced growth and rot of carrots by *Sclerotinia* were characteristic of calcium nitrate and Nitro-9 washes, especially in 1999 tests (Figure 2.b.3, c and d).

The complete inhibition of sclerotinia rot by continuous cold storage at 1 °C is consistent with the low cold tolerance reported previously for *S. sclerotiorum* in stored carrots (Finlayson *et al,* 1989b). Inhibition of Sclerotinia rot by calcium propionate and sodium hypochlorite washes is consistent with the reported inhibitory effect of these washes on black rot (*Thielaviopsis basicola*) in stored carrots (Punja and Gaye, 1993). The elevated temperatures in storage and the use of washes such as Nitro-9 that increase the pH to10 on the carrot surface enhances the growth and decay by soft rot bacteria (*Erwinia carotovora*). Extensive internal bacterial soft rot at the sites on mechanical harvest wounds and sites of rootlet emergence confounded the tests of chemical washes at temperatures above 1°C.

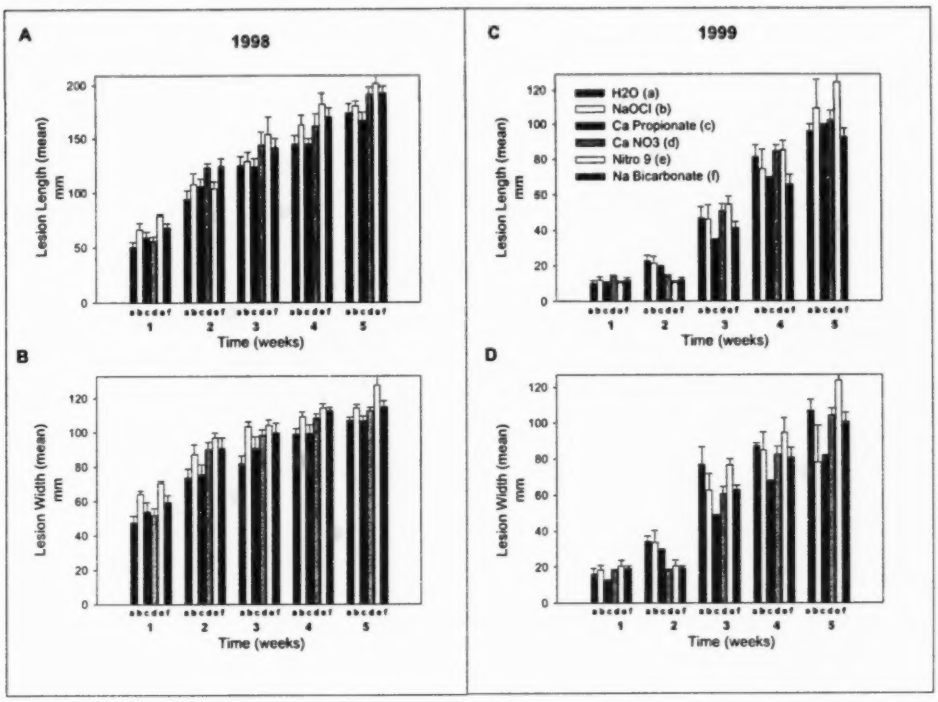

Figure 2.b.3. Mean lesion lengths (mm) and widths (mm) on stored carrots for three replicate boxes treated with washes of check distilled water **(a)**, 100 ppm sodium hypochlorite **(b)**, 1 % calcium propionate **(c)**, 0.1 % calcium nitrate **(d)**, 1 % calcium in Nitro-9 solution **(e)** and 1 % sodium bicarbonate **(f)**, wound inoculated mid-point on the centre carrot of five carrots placed side-by-side in plastic crisper boxes lined with moist paper and incubated for 8 weeks at 1 and 6 °C randomly positioned on shelves in cold storage rooms. The experiments were conducted in the fall-winter of 1998 (A and B) and 1999 (C and D). Bars represent standard errors of the means.

2.c Field evaluation of the efficacy of the bio-control agent *Trichoderma harzianum* for the control of sclerotinia rot in field and stored carrots

A) Root Colonization

T. harzianum colonized the carrot roots early in the growing season, however, the overall persistence of colonization rate decreased over time. A significant difference was found between all T-22 treatments and the untreated check in the 1st (26 July) (P=0.0007) and 3rd (5 November) (P=0.0063) assessment intervals, however, no significant differences were observed among the T-22 treatments (Table 2.c.1). An overall significant effect of the assessment interval was observed (P=0.0019) suggesting a decline of carrot root colonization rate by *T. harzianum* over time.

The highest level of root colonization rate was provided by the T-22D at 10 kg/ha treatment at the 1st assessment interval (80.2%), however it dropped at the 2nd assessment interval (34.7 %) and remained almost stable until harvest. The T-22G at 60 kg/ha provided numerically the lowest root colonization rate at the 1st assessment interval (52.1%), and the highest rate among all T-22 treatments at harvest (45.8%). This indicated that T-22G at 60 kg/ha performed better by providing root colonization in a long run. The dry weather during the 2nd assessment (26 August)may have had an adverse effect on the activity of *T. harzianum,* explaining the slight drop in the colonization rate.

Table 2.c.1. Temporal root colonization rates by *Trichoderma harzianum* in carrots grown in organic soil of Muck Crop Research Station, Bradford-Holland Marsh, Ontario, treated with low and high rates of drench and granular formulations of biofungicides,T-22 RootShield Drench and T-22 RootShield Granular, in 1999.

			Carrot root colonization over time (%)		
Treatments	**Rate kg/Ha**	**Application method**	**36366**	**36397**	**36468**
Untreated Check	-	-	6.3c* (a)**	13.9a (a)	6.9b (a)
T-22D	10	Drench	80.2a (b)	34.7a (b)	36.1a (b)
T-22D	20	Drench	64.6a (b)	34.7a (ab)	43.1a (b)
T-22G	30	Granular	53.1ab (b)	37.5a (a)	37.5a (a)
T-22G	60	Granular	52.1ab (b)	23.6a (ab)	45.8a (b)

* Numbers in a column followed by the same letter are not significantly different at Type I error rate $\alpha = 0.05$.

** Numbers in a row followed by the same bracketed letter, are not significantly different at Type I error rate $\alpha = 0.05$ (Fisher's Protected LSD Test)

B) Sclerotinia rot development in storage

The experiment was still in progress at the end of this project, however data from the first assessment are presented in Table 2.c.2. Two months after harvest there were no differences in disease incidence, but differences in severity were identified (Table 2.c.2.). A significant increase in disease severity was observed by the T-22 Drench applied as a post-harvest dip (P=0.0097) in comparison to the rest of T-22 treatments. About the same measure of protection was provided by both application rates of T-22 Granular and T-22 Drench at 10 kg/Ha. This suggested that the carrot wash might have adversely affected the protection that the soil along with its living micro-organisms provided to the carrot root in addition to the introduced beneficial fungus, *T. harzianum*. *T. harzianum* did not provide significant effect in the reduction of sclerotinia rot disease incidence and severity on stored carrots, two moths after the artificial inoculation.

Table 2.c.2. Sclerotinia rot incidence and severity in carrots treated with *Trichoderma harzianum* based biofungicides, and stored in cold temperature controlled Filacell storage at 1.0 ± 1.00C and 90 ± 5% relative humidity, two months after artificial inoculation with *S. sclerotiorum*, in 1999-2000.

			Sclerotinia rot ratings	
Treatments	**Rate kg/Ha**	**Application method**	**Disease incidence (%)**	**Disease severity index**
Check	-		22.50a*	27.33ab
T-22 Drench	10	Drench	15.00a	24.17b
T-22 Drench	20	Drench	20.00a	26.50b
T-22 Granular	30	Granular	15.83a	24.17b
T-22 Granular	60	Granular	13.33a	24.00b
T-22 Drench	8.6 g/100 ml H_2O	Post-harvest dip	34.17a	31.83a

* Numbers in a column followed by the same letter are not significantly different at Type I error rate α=0.05 (Fisher's Protected LSD Test).

C) Yield assessment

There were no differences in yield of the carrots treated with high doses of both granular and drench treatments compared to the untreated check (Table 2.c.3).

Table 2.c.3. Effect of *Trichoderma harzianum* on the total and marketable yield of carrots grown in organic soils of Muck Crop Research Station, Bradford-Holland Marsh, Ontario, treated with low and high rates of drench and granular formulations of bio-fungicides, T-22 Rootshield Drench (T-22D) and T-22 RootShield Granular (T-22G), in 1999.

			Yield ratings	
Treatment	**Rate kg/Ha**	**Application method**	**Total T/Ha**	**Marketable** (%)**
Untreated check			83a*	96.6a
T-22D	10	Drench	85a	94.5a
T-22D	20	Drench	94a	94.5a
T-22G	30	Granular	86a	95.8a
T-22G	60	Granular	95a	96.6a

* Numbers in a column followed by the same letter are not significantly different at Type I error rate α=0.05 (Fisher's Protected LSD Test).

** In marketable yield, split and forked carrots were graded out.

D) Cavity spot assessment

Cavity spot disease, caused by *Pythium* spp., is highly prevalent in carrots grown in organic soils. In 1999 the disease pressure was particularly severe and the cavity spot incidence reached extremely high levels in almost all treatments. No significant differences were observed among the treatments in either disease incidence or disease severity ratings. (Table 2.c.3).

Table 2.c.4. Effect of *T. harzianum* on the cavity spot disease incidence and severity index in carrots grown in organic soils of Muck Crop Research Station, Bradford-Holland Marsh, Ontario, treated with low and high rates of drench and granular formulations of bio-fungicides T-22 Rootshield Drench (T-22D) and T-22 Rootshield Granular (T-22G), in 1999.

			Cavity spot ratings	
Treatment	**Rate kg/Ha**	**Application method**	**Disease Incidence (%)**	**Disease severity Index**
Untreated check			90.00a*	41.75a
T-22D	10	Drench	96.25a	43.25a
T-22D	20	Drench	88.75a	35.75a
T-22G	30	Granular	83.75a	35.75a
T-22G	60	Granular	85.42a	33.75a

* Numbers in a column followed by the same letter are not significantly different at Type I error rate $\alpha = 0.05$ (Fisher's Protected LSD Test).

2.d Assessment of the survival of sclerotia of *S. sclerotiorum* exposed to the bio-control agent *T. harzianum*

A) Sclerotia colonization

The highest levels of sclerotia colonization by *T. harzianum* were observed two weeks after the sclerotia were buried into the soil treated with T-22 Drench at 20 kg/ha (45.83%), T-22 Drench at 10 kg/ha (22.50%) and T-22 Granular at 30 kg/ha (22.50%). However, T-22 Drench at 20 kg/ha treatment only had a significantly higher percentage of colonized sclerotia compared to T-22G at 60 kg/ha (4.17%) and the control (1.67%) treatments. No significant differences were observed among treatments in the following 4th, 6th, 8th, 10th and 12th weeks, although is interesting to point out that in three consecutive time intervals (6th, 8th, 10th week assessments), T-22 G at 60 kg/ha was found superior to other treatments.

The colonization rate of sclerotia by *T. harzianum*, appears to decrease over time for almost all treatments, with a slight increase between the 8th and the 10th weeks after burial. Apparently the drench is more readily available immediately after application compared to the granular.

Table 2.d.1. Colonization rate by *T. harzianum* of sclerotia of *S. sclerotiorum* buried in organic soil of Muck Crop Research Station, Bradford-Holland Marsh, Ontario, treated drench and granular formulations of bio-fungicides, T-22 RootShield Drench (T-22D) and T-22 RootShield Granular (T-22G), in 1999.

Treatment	Rate kg/Ha	Application method	Sclerotia colonization in weeks after burial (%)					
			2	4	6	8	10	12
Untreated check			1.7b*	1.7a	0.0a	0.0a	2.5a	6.7a
T-22D	10	Drench	22.5a b	11.7a	0.8a	2.5a	7.5a	0.8a
T-22D	20	Drench	45.8a	9.2a	5.8a	1.7a	4.2a	0.8a
T-22G	30	Granular	22.5a b	6.7a	1.7a	0.8a	1.7a	2.5a
T-22G	60	Granular	4.17b	5.0a	9.2a	4.2a	10.8a	3.3a

* Numbers in a column followed by the same letter are not significantly different at Type I error rate $\alpha = 0.05$.
(Fisher's Protected LSD Test)

The soil was moderately dry during seeding (spring 1999) but there was sufficient moisture for the carrot seed to germinate. Assessment of colonizationlevels of T-22 G suggests that T-22 granular at 60 kg/ha behaved differently from other formulations and rates. T-22 granular at 60 kg/ha has the lowest level of colonization rate (4.17%), increases to 9.17% after the 6th weeks and maintains a certain stability for up to the 10th week, with the exception of a drop in the 8th week. Between the 6th and the 8th week of assessment, it was a particularly dry weather which might have adversely affected the performance and the activity of *T. harzianum*. However, among all T-22 treatments, T-22 G at 60 kg/ha, provided the best sclerotial colonization rate in the long run, from the 6th to the 12th week after the burial. Low levels of colonization by *Trichoderma* spp. (1.67%), were observed also in control sclerotia from 2 weeks after burial. This appears to be more evident between the 10th and the 12th weeks when the colonization rate overpasses all T-22 treatments (6.67%). The fact that the colonization rate of control sclerotia increases over time, suggests that, there may have been interplot interference, or naturally occurring *Trichoderma* spp. in the soil since both formulations were applied locally in the furrow. Naturally occuring Trichoderma spp. have been found in these soils and on Sclerotium cepivorum.

B) Sclerotia recovery

The recovery rate of sclerotia also followed a decreasing pattern over time, for all the treatments including the control. In a twelve week period, the recovered sclerotia decreased from 80% to about 26%. Although there were no significant differences among treatments for each

week, a significant time effect was observed among assessment intervals. This suggests that the time factor is of great importance in sclerotia survival. Significant differences are found between the 2nd week and all the following time intervals as well as between the 4th and 6th week and the 8th, 10th and 12th week treatments (Table 2.d.2). However, there is no indication that exposure to soil infested with T-22 affects the survival of the sclerotia.

There are numerous interactive natural factors including environmental and biological (naturally occurring micro-flora and micro-fauna) that affect the persistence and survival of sclerotia in soil. Further investigation is needed to determine what factors affect the disintegration of sclerotia in soil.

Table 2.d.2. Recovery rate of sclerotia of *S. sclerotiorum* buried in organic soil of Muck Crop Research Station, Bradford-Holland Marsh, Ontario, treated with drench and granular formulations of biofungicides, T-22 RootShield Drench (T-22D) and T-22 Biological Plant Protectant Granules (T-22G), in 1999.

Treatment	Rate kg/Ha	Application method	Sclerotia recovery in weeks after burial (%)					
			2	4	6	8	10	12
Untreated check			69.2a*	55.0a	50.0a	28.3a	18.3a	25.8a
T-22D	10	Drench	81.7a	60.0a	44.2a	29.2a	25.0a	26.6a
T-22D	20	Drench	80.0a	46.7a	44.2a	30.0a	29.2a	25.8a
T-22G	30	Granular	78.3a	52.5a	60.0a	37.5a	23.3a	29.2a
T-22G	60	Granular	82.5a	65.8a	47.5a	32.5a	35.0a	26.7a

*Numbers in a column followed by the same letter are not significantly different at Type I error rate $\alpha = 0.05$. (Fisher's Protected LSD Test)

C) Sclerotia viability

The viability of sclerotia, decreased over time, but was not affected by treatment. There were 10-13% viable sclerotia in the 2nd week and this dropped to 0-4% in the 4th week. Although there were no significant differences between treatments for each week, a significant time effect was observed between assessment intervals. This suggests that time is an important factor in sclerotial viability. Significant difference were observed only between the 2nd week treatment and the rest of the following time intervals (Table 2.d.3). There is an unexplained phenomenon of a slight increase of sclerotia viability from the 10th to the 12th week for three treatments including the check. A considerable level of contamination was noted on plated sclerotia by various soil-borne fungi such as *Mucor* spp., *Rhizopus* spp. and *Fusarium* spp. However, in almost all occasions

where present, the aggressive *T. harzianum* was able to quickly outgrow the contaminants in the plate.

Table 2.d.3. Viability rate of sclerotia of *S. sclerotiorum* buried in organic soil of Muck Crop Research Station, Bradford-Holland Marsh, Ontario, treated with drench and granular formulations of the *T. harzianum* fungus based biofungicides, T-22 RootShield Drench (T-22D) and T-22 RootShield Granules (T-22G), in 1999.

Treatment	Rate kg/Ha	Application method	Sclerotia viability in weeks after burial (%)					
			2	4	6	8	10	12
Untreated check			2.5a	0.8a	0.0a	0.0a	0.8a	1.6a
T-22D	10	Drench	4.2a	0.0a	0.8a	0.0a	0.0a	0.0a
T-22D	20	Drench	3.3a	0.8a	2.5a	0.0a	0.8a	4.2a
T-22G	30	Granular	13.3a	3.3a	0.8a	0.0a	0.8a	4.2a
T-22G	60	Granular	10.0a	2.5a	0.8a	0.0a	0.0a	1.7a

* Numbers in a column followed by the same letter are not significantly different at Type I error rate $\alpha = 0.05$. (Fisher's Protected LSD Test).

2.e Monitoring micro-climate, pathogen and disease incidence data for the epidemiological study of sclerotinia rot of carrots

A) Monitoring environmental variables

Carpogenic germination of sclerotia and emergence of apothecia, in both sites, was preceded by relatively cool soil temperatures and heavy rainfalls. The two week period prior to this event in Site 1 (15 September), was characterized by a mean soil temperature of about 19 °C and high soil moisture contents ranging from 41- 42% (v/v) (Fig. 3). The week prior to the event in Site 2 (12 August), was characterised by soil temperatures ranging from 13°C to 18°C, and soil moisture content ranging from 37-38% (v/v) (Fig.4). Apparently, the high soil moisture content and the earlier full canopy closure incited the emergence of apothecia earlier in Site 2 than in Site 1.

The third week prior to the disease onset in the field (18 September) in Site 2 was characterized by air temperatures ranging from 19-21°C and air relative humidity ranging from 79-97%, whereas the soil moisture remained at moderate levels (31% v/v). Although no rainfall was recorded during this week, the heavy dew caused long durations of leaf wetness periods, particularly on the exposed leaves. The first and the second weeks prior to the disease onset were characterized by heavy rainfall (72.2 mm in total) which increased the soil moisture close to field capacity levels (40-42% v/v) and leaf wetness duration levels.

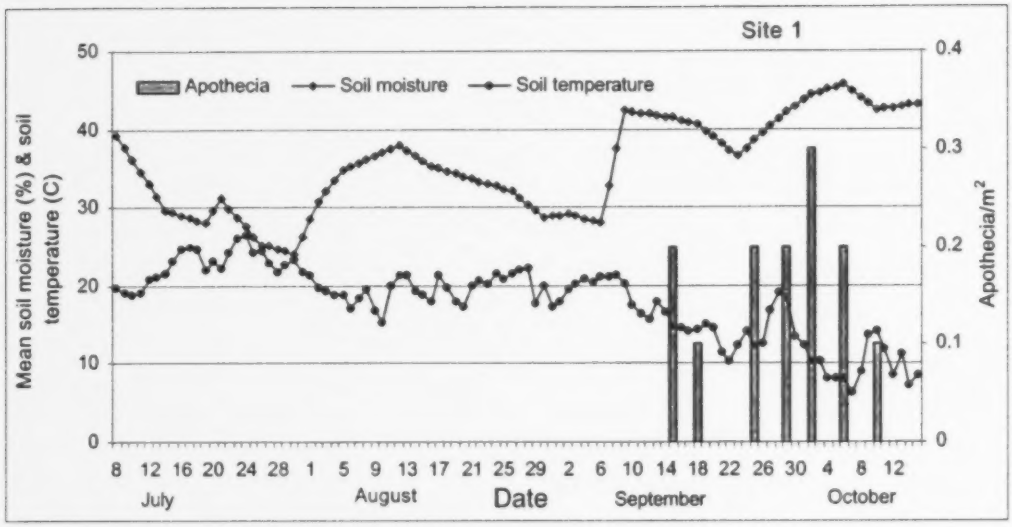

Figure 3. Emergence of apothecia in relation to mean soil moisture and soil temperature in site1, at Muck Crop Research Station, Bradford-Holland Marsh, Ontario, during summer 1999.

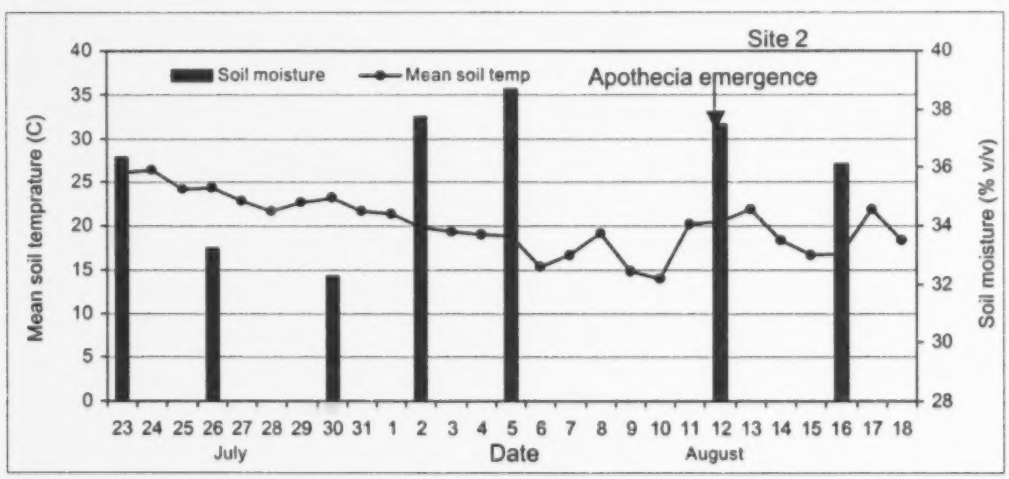

Figure 4. Emergence of apothecia in relation to mean soil moisture and soil temperature in site 2, at Bradford-Holland Marsh, Ontario, during summer 1999.

B) Monitoring pathogen's variables

In Site 1, apothecia were first detected on 15 September at the density level of 3 apothecia/16 m linear or carrot row, and reached a peak of 5 apothecia/16 m linear on 2 October. Air-borne ascospores within the crop were first observed at very low concentration (0.01 CFU/cm^2 of media plate) on 16 August, and multiple spore release events were observed during the following 2 months. The first peak of ascospore discharge within the crop was observed on 18 September (0.51 CFU/m^2 of media plate) and a second one (0.77 CFU/cm^2 media plate) on 10 October, corresponding with the peaks of apothecial density within the plot (Fig.5). The observation of ascospores about three weeks prior to the emergence of apothecia, suggested the existence of a source of air-borne inoculum from outside the plot.

In Site 2, both carpogenic germination of sclerotia and ascospore release appeared to occur about three weeks prior to site 1. Apothecia were observed only once, on 12 August, at the density of 4 apothecia/16 m linear of carrot row. Ascospores within the crop were first detected on 9 August and multiple spore release events were also observed during the following 2 months. There first peak of ascospores discharge (1.22 CFU/cm^2 of media plate) was observed on 23 August and a second one (0.45 CFU/cm^2 media plate) on 18 September (Fig. 6). Increase of ascospore concentration, appeared to correspond with the emergence of apothecia within the crop.

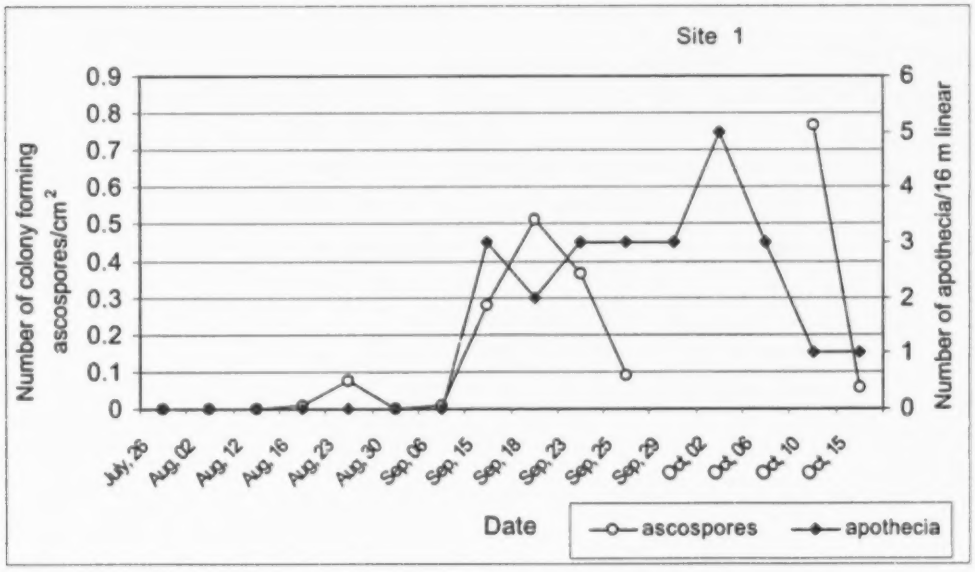

Figure 5. Presence of colony forming ascospores and apothecia emergence over time in site 1, at Muck Crop Research Station, Bradford-Holland Marsh, Ontario, during summer 1999. (Note missing ascospore data for the period 29 Sept - 06 Oct because continuous rainfall interfered with the ascospores monitoring technique).

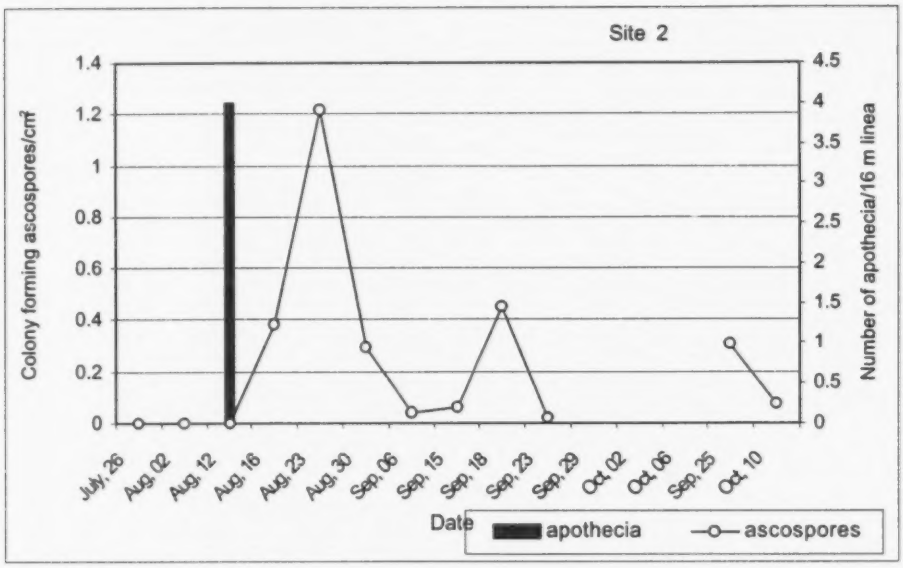

Figure 6. Presence of colony forming ascospores and apothecia emergence over time in Site 2, at Bradford-Holland Marsh, Ontario, during summer 1999.

For both sites, ascospore density data are missing for the period of 29 September - 06 October, because continuous rainfall interfered with the ascospore monitoring technique. Canopy density appeared to have an important effect on the pathogen's life cycle. In site 2, where the carrot rows were closer (72 cm apart) than in site 1 (86 cm apart), the canopy was denser and completely closed by mid July, creating thus a favorable micro-climate for the pathogen to complete important biological events, such as the germination of sclerotia, earlier in the season.

C) Monitoring disease incidence in the field

In Site 1, no disease occurred during the growing season in the field.

In Site 2, the first foliar sub-samples that tested positive for the presence of the *S. sclerotiorum* during pre-symptomatic assessments, were detected on 26 August. During the pre-symptomatic phase, no visible lesions of sclerotinia rot disease were observed on the sampled plants.

The foliar infection increased rapidly after 13 September and the first disease symptoms in the field were observed on 18 September (16%). Infection incidence reached a maximum of about 24% on 25 September and then decreased to 19% during the first half of October (Fig. 7). Disease started after a long period of heavy rainfall which increased soil moisture and leaf wetness duration levels. Most of the month of August was characterized by very heavy dew and high

relative humidity levels. The carrot foliage was wet for over 14 hours per day (from 4:00-5:00 pm - 10:00-11:00 am). Foliar infection also progressed substantially during long periods of rain. Total rainfall for the month of September reached 137 mm, which was above the average of 10 years.

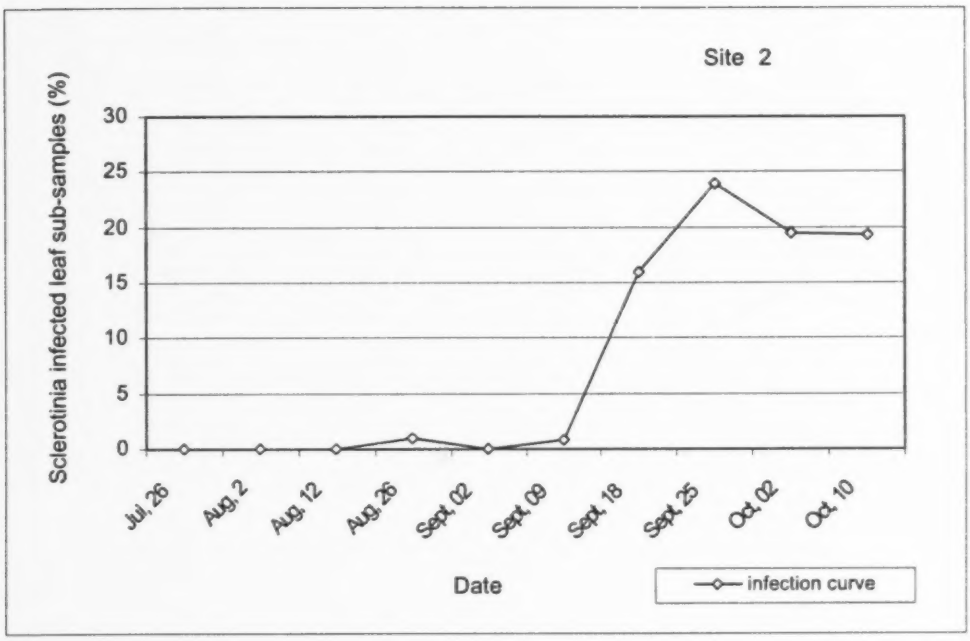

Figure 7. Foliar infection curve over time in Site 2, at Bradford-Holland Marsh, Ontario, during summer 1999.

Apparently, the ascospores within the crop landed on the carrot foliage but may have remained quiescent until favorable conditions for infection occurred. Typical symptoms of foliar infection detected in the field were water-soaked dark olive-green lesions on leaves, petioles and leaflet joints, followed by a white cottony aerial mycelium growing on the infected tissues. Large, black sclerotial bodies were formed among the mycelium shortly after the decay of the leaf tissue. The leaves that showed visible disease symptoms in the field, were mostly the senescing ones that were in contact with the soil (attached or detached from the carrot plant) or the young and/or senescent leaves that bent over and contacted the infected leaves laying on the ground. Almost 50% of the leaflet and petiole sub-samples from the ground leaves tested positive for sclerotinia infection as opposed to 8 or 6 % respectively from young and senescent upright leaves. The ground leaves were also the major contributors to the total amount of disease in the field, as they provided 84-86% of the total sub-samples that tested positive for sclerotinia infection. Abawi and Grogan (1979) reported that infection of bean blossoms by ascospores occurred after 48-72 hours of continuous wetness, whereas Finlayson (1989a) reported that leaf wetness had to be maintained

for 11 consecutive days for foliar applied ascospores to induce disease symptoms on carrot foliage. It is highly probable that the ascospores that land on the senescent ground leaves have better chances to reach the required combination of favorable micro-climatic conditions of cool temperature, free water (since they are in continuous contact with the wet soil) and ready available exogenous nutrients. The organic muck soils in particular retain water for a long time and the water evaporation rate is also slowed down because of the closed dense canopy. The upright leaves undergo a periodic wet and dry condition during the day/night cycle, thus limiting the infection cycle.

The infection progressed through the petioles toward the crown of the carrot, however, at harvest, diseased carrot roots were hardly visible. No infection was detected on carrot roots as a result of direct penetration of hyphae originated from sclerotia that germinated myceliogenically in the soil during the growing season. The first disease incidence at carrot root level (0.25%) was detected after the shoot had shown disease symptoms (10 October). The detection was made possible through the plating technique as no visible symptoms were noted.

D) Monitoring survival and viability of sclerotia buried in organic soil over time

A significant difference was found for both recovery and the viability of the sclerotia overwintered in organic soil compared to the check (sclerotia stored in lab at 4□C for the entire experiment period), for every time interval examined, except for the recovery rate at first assessment (15 April). A decreasing pattern is observed for both recovery and the viability rates of sclerotia buried into the soil over time, but viability suffered the most severe decrease. A significant decrease in survival rate (from 82.5% to 17.5%) was observed at the second assessment interval, (5 May), immediately after the soil was thawed and when the increasing temperatures triggered the activity of the micro-organisms in soil. The viability was drastically reduced since the first assessment, in early spring (6.6%) and did not change during the growing season. The viability rate of the check sclerotia ranged between 73.3% to 91.6%, with no significant differences between assessment intervals (Fig. 8).

A high disintegration rate was observed in buried sclerotia over time as result of the time-soil treatments interaction effect. When recovered, the sclerotia residues were colonized by numerous microorganisms, including mites (*Rhizogliphus* spp.), nematodes, and spring-tails (*Folsomia* spp.). When and plated on culture media, the surface sterilized sclerotia were colonized by numerous soil-borne fungi, where the most prevalent were *Mucor* spp., *Rhizopus* spp., *Penicillium* spp. and *Fusarium* spp. It is highly probable that these soil inhabitants use sclerotia as food source and in combination with the fluctuating soil environmental factors (temperature, moisture, pH, salt and nutrients) contribute to the inoculum reduction in soil over time. However, the naturally produced sclerotia in soil and sclerotia formed on diseased plant tissues which return to the soil after harvest, compensate to a certain degree the natural disintegration, contributing thus to the overall increase of inoculum in soil.

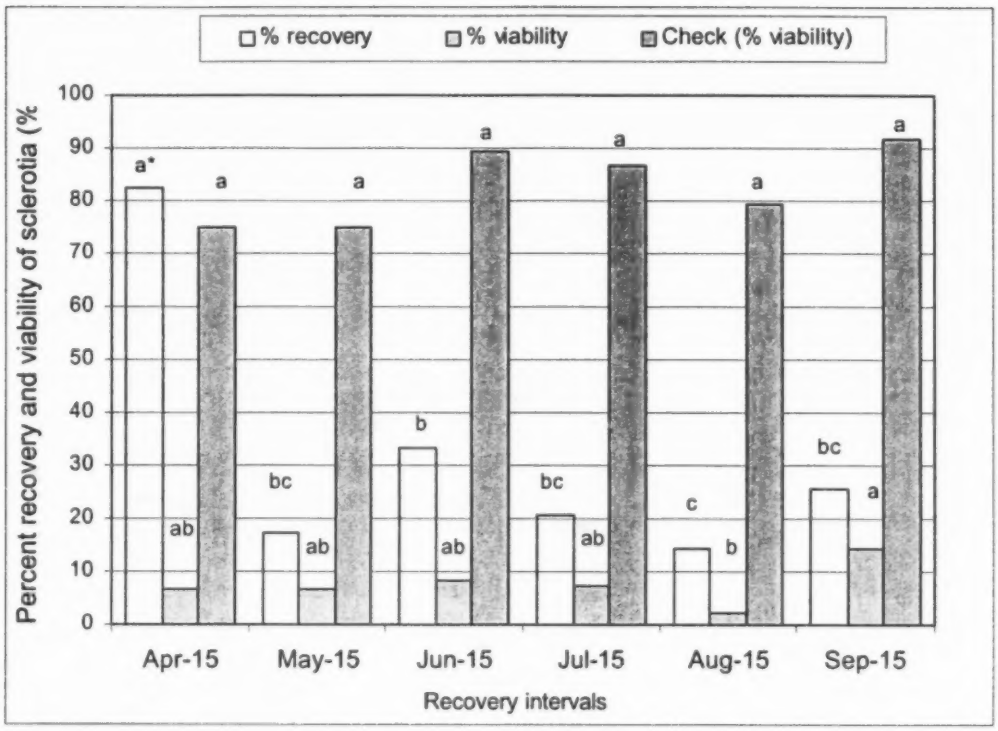

Figure 8. Recovery and viability rate of sclerotia buried in soil over time in Site 1, at Muck Crop Research Station, Bradford-Holland Marsh, Ontario, during summer 1999. (* The same patterned bars labelled with the same letter are not significantly different at □= 0.05, Fisher's Protected LSD Test).

C) Potential integration of control methods in onion and carrot production

A) Assessment of potential integration of DADS applications with cut and peel carrots and DADS residue determination on carrot roots

Two separate taste test took place on consecutive days. No significant differences were found between test, therefore the data were pooled for analysis. Significant differences were observed among cultivars in sweetness, overall carrot flavour, preference and sugar content. Both the DADS and check treatment scored significantly better for sweetness, overall carrot flavour and preference that the other cultivars in the taste test. There was significant differences found in the sugar content, but not between the DADS and check treatment. It is clear that growing carrots in soil treated with DADS has no affect on taste of sugar content.

Table 3.a.1. Summary of Carrot Taste Test on Carrots grown in DADS treated soil.

Treatment	Harsh, Biting Flavour - A	Sweetness - B	Overall Carrot Flavour - C	Preference - D	Sugar Content *	DADS Residue
Check	2.75 a **	4.00 ab	4.75 a	4.58 ab	9.83 b	ND
DADS	2.75 a	5.00 a	4.33 ab	5.67 a	10.17 b	ND
Nantes	4.00 a	3.08 bc	3.00 c	2.58 c	8.83 c	
Fontana	3.67 a	2.75 c	3.08 c	2.91 c	8.83 c	
Cellobunch	3.50 a	3.83 bc	3.33 bc	3.58 bc	11.33 a	

Flavour Scale = A - 1 = lacking in harshness or biting flavour
7 = strong burning flavour, harsh, turpentine, or carrot top - like
B - 1 = imperceptible
7 = extremely sweet
C - 1 = weak carrot flavour
7 = strong carrot flavour
D - 1 = dislike extremely
7 = like extremely

* Sugar content measured in Brix (percent soluble solids).

** Numbers in a column followed by the same letter are not significantly different at P = 0.05, Fisher's Protected LSD Test

ND = Not detectable

CONCLUSIONS

4. Management of Onion White Rot

Synthetic materials that stimulate germination of the sclerotia of *S. cepivorum* can provide effective control of onion white rot. Diallyl disulfide (DADS) was most effective, especially when applied to a field twice before onions were grown. N-dipropyl disulfide (DPDS) was not as effective in reducing white rot as was DADS. Two application of DPDS were required to obtain any reduction in the incidence of white rot of onions. This work demonstrated that DADS application can be an effective management method for onion white rot, at least in years where disease pressure is not extreme.

Host resistance to onion white rot is another potentially effective method of managing this disease. Differences in white rot resistance were identified and have been incorporated into the breeding programs of commercial onion breeders. A scale inoculation technique, which provides a highly reproducible and more efficient method of screening for resistance was tested. This method can be used to provide a pre-screening method before selected breeding lines are tried in

the field. *T. harzianum*, in the commercial formulation T-22, was not effective in reducing the survival of sclerotia of *S. cepivorum*, under the test conditions.

5. Management of Sclerotinia Rot of Carrot

This research has identified two new and important factors in the epidemiology of sclerotinia rot of carrot. First, senescent and decaying leaves laying on the ground are the major contributors to foliar stage of disease in the field. It is highly probable that senescent leaves attached to the plant, in particular, are the major transmitters of the infection to the carrot tap root through the crown. Secondly, the chopped infected tops left behind in the field after harvest, may contribute to the increase of the inoculum in soil over the years and consequently may be considered one of the most significant factors in the carryover of the disease.

The epidemiology of sclerotinia rot on carrots starts with the carpogenic germination of sclerotia within the field. These are the major source of the air-borne inoculum responsible for the foliar infection. Sclerotinia rot appears in the carrot field close to the end of the growing season at a time when carrot plants are close to maturity. Then the disease progresses very rapidly, assisted by the typical cool and wet weather in the fall.
Soil moisture and soil temperature appeared to be the most important environmental variables for both carpogenic germination of sclerotia and disease onset and progress in the field.

Sclerotia buried in the soil undergo a natural disintegration process that appears to be detrimental to their physical survival and viability. There was evidence that the bio-control fungus *T. harzianum,* colonized sclerotia buried in treated soil, however a significant decrease of its persistence was observed over time. No effect of *T. harzianum* was observed on the decrease of physical survival and viability rate of sclerotia buried in soil, under the test conditions. Similarly, there was evidence of carrot root colonization by *T. harzianum* after emergence, however the persistence of the bio-control was observed to decrease over time. Finally, none of the treatments had any effect on disease development and severity in storage.

Controlled environment trials with post-harvest treatments demonstrated that storage at 1□C provided the best suppression of sclerotinia rot, in the absence of any treatments. However, growers are not always able to control the temperature in commercial storage to prevent outbreaks of disease. In the event that the storage temperature increases, disease can develop rapidly and no post-harvest treatments are effective for disease control.

1. Potential integration of disease management on onions and carrot production

Improved management methods for soil borne diseases will allow growers to make better use of the many advantages of crop rotation. Onions and carrots are good rotational crops.

There was no negative effect of DADS treatment on a subsequent crop of carrots grown for cut-and-peel processing. Therefore, including these carrots in a rotation to allow for treatment of a field with DADS for white rot control should be possible and could be recommended once DADS is registered. Incorporating cut-and-peel carrots, which have a shorter growing season, allows for spring and fall application of DADS in the same year.

The commercial formulation of *T. harzianum* was not effective in controlling the sclerotia of *S. cepivorum* or *S. sclerotiorum* under the conditions of these trials.

FUTURE DIRECTIONS

Optimizing the control of soil borne diseases of onions and carrots to improve quality, storability, and effective crop rotation, remains an issue, although the research reported here certainly is a significant step toward improved disease management, especially for onions.

Diallyl disulfide is an effective management method for onion white rot and can be used in rotation with carrots, especially those grown for the cut-and-peel industry. However, United Agri-Products has not yet submitted the registration package for DADS to the PMRA. In the United States, DADS is considered a bio-rational material, eligible for "fast-tracking" for registration through the Environmental Protection Agency. Thus, UAP has chosen to pursue registration of DADS in the US before submitting it for registration in Canada, in case that the Canadian system would slow a joint registration. This has left growers with no access to DADS for onion white rot management.

There are some natural alternatives to DADS, which may also stimulate germination of sclerotia of *S. cepivorum.* Several natural products of garlic contain varying levels of DADS. Garlic juice, made from the scapes of garlic, is available in Ontario. Garlic oil and garlic powder, both produced for human consumption, are available from the United States. Growers have also asked whether cull onions could be juiced and the juice used to treat infested soil. Trials to test these products for the ability to reduce white rot in the field and to stimulate germination of sclerotia under controlled conditions have been initiated and should be pursued further.

Resistance in onions to white rot, even if partial, would be a major improvement in disease management. Screening onions for resistance in the field is continuing at the Muck Crops Research Station. Marilyn Hovius, who did much of the onion white rot work for this project, is now pursuing a Ph.D. program at the University of Wisconsin, to study mechanisms of resistance and further develop a rapid laboratory methods to screen onions for resistance.

Although the formulations of *T. harzianum* used in these trials were not effective in controlling *S. cepivorum* or *S. sclerotiorum* under the test conditions, *T. harzianum* is known to be effective for the biological control of many soil borne diseases. Research should be conducted to determine if the efficacy of *T. harzianum* could be improved through manipulating the environment (ie. soil moisture) or application methods.

The research on the management of sclerotinia rot on carrots has raised several important questions about the epidemiology and control of the disease. Further research needs to be conducted (some is underway) to confirm the observations on infection and disease development in the field, including the role of soil contact in providing moisture for infection of leaves, and the contribution of sclerotia from infected leaves and leaf debris in increasing soil inoculum for following years. These also raise practical questions for disease management about the possibility of manipulating the crop to reduce the number of senescent leaves that come into contact with the soil, and managing crop debris to reduce or eliminate an increase in the populations of sclerotia in soil.

Finally, it is clear that improved methods are needed to reduce the development and spread of sclerotinia rot in cold storage. Certainly, reducing infection in the field is important, however a few infected carrots in storage can cause high levels of infection if storage conditions vary from ideal. Post-harvest fungicide treatments are not acceptable because of the risk of high residues, therefore, biological or bio-rational approaches are indicated. As a direct result of the research funded by this program, two graduate students, Cezarina Kora (Ph.D) and Maria Jaime (MSc.) are currently working on these issues at the University of Guelph, both with NSERC funding. A third individual, Marilyn Hovius, is pursuing a Ph.D. at the University of Wisconsin.

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